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Synthesis, SAR study, and biological evaluation of novel quinoline derivatives as phosphodiesterase 10A inhibitors with reduced CYP3A4 inhibition

Wataru Hamaguchi *, Naoyuki Masuda, Satoshi Miyamoto, Yasuhiro Shiina, Shigetoshi Kikuchi, Takuma Mihara, Hiroyuki Moriguchi, Hiroshi Fushiki, Yoshihiro Murakami, Yasushi Amano, Kazuya Honbou, Kouji Hattori

Drug Discovery Research, Astellas Pharma Inc., 21, Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

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ABSTRACT

A novel class of phosphodiesterase 10A inhibitors with potent PDE10A inhibitory activity and reduced CYP3A4 inhibition was designed and synthesized starting from 2-[4-({[1-methyl-4-(pyridin-4-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline (**1**). Replacement of pyridine ring of **1** with *N*-methyl pyridone ring drastically improved CYP3A4 inhibition, and further optimization of these quinoline analogues identified 1-methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)phenoxy]methyl}-1*H*-pyrazol-4-yl)pyridin-2(1*H*)-one (**42b**), which showed potent PDE10A inhibitory activity and a good CYP3A4 inhibition profile. A PET study with ^{11}C-labeled **42b** indicated that **42b** exhibited good brain penetration and specifically accumulated in the rodent striatum. Further, oral administration of **42b** dose-dependently attenuated phencyclidine-induced hyperlocomotion in mice with an ED_{50} value of 2.0 mg/kg and improved visual-recognition memory impairment at 0.1 and 0.3 mg/kg in mice novel object recognition test.

1. Introduction

Schizophrenia is a chronic and debilitating psychiatric disorder that affects approximately 1% of the world's population.[1] However, the majority of current therapeutic treatments primarily address positive symptoms, with only limited efficacy on negative symptoms and cognitive dysfunction. In addition, current antipsychotics frequently cause undesirable side effects such as extrapyramidal syndrome, weight gain and diabetes,[2] highlighting the unmet medical needs for drugs less prone to such side effects.

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that regulate intracellular signaling by hydrolyzing cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The PDE superfamily of enzymes is divided into 11 families in mammals (PDE1–11). PDE10A enzyme is a dual substrate (cAMP/cGMP) phosphodiesterase that is highly expressed in the brain, particularly in the medium spiny neurons of the mammalian striatum.[3] The striatal complex forms the core of the basal ganglia, a system of interconnected nuclei that process cortical information in the context of dopaminergic signaling to regulate motoric, appetitive, and cognitive processes.[4] Inhibition of PDE10A may enhance the intracellular second messenger signaling and striatal output suggested to be impaired in schizophrenic patients.[5] Further, some selective PDE10A inhibitors such as MP-10 (Fig. 1) have shown potent efficacy in several rodent behavioral models of schizophrenia.[6] Thus, PDE10A inhibitors have garnered attention as a new therapeutic method for the treatment of schizophrenia.[7]

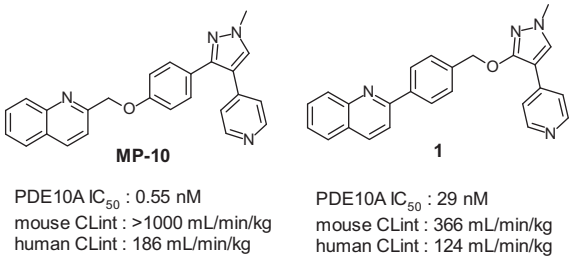

Figure 1. Structures of MP-10 and compound **1**.

We previously reported that the quinoline analogue **1** (Fig. 1) had moderate PDE10A inhibitory activity with in vivo efficacy in mice behavioral model for positive symptom and cognitive impairment of schizophrenia.[7r] Compound **1** also exhibited improved metabolic stability in human liver microsomes (HLM),

* Corresponding author. Tel.: +81 29 863 6743; fax: +81 29 852 5387.
E-mail address: wataru.hamaguchi@astellas.com (W. Hamaguchi).

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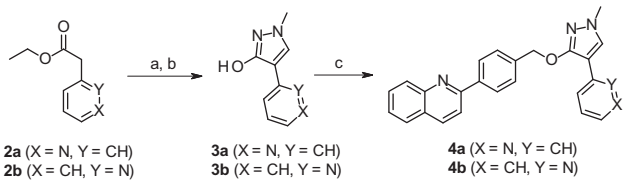
0968-0896/



Scheme 1. Reagents and conditions: (a) *N*,*N*-dimethylformamide dimethyl acetal, DMF; (b) methylhydrazine, acetic acid, EtOH; (c) [4-(quinolin-2-yl)phenyl]methanol, ADDP, nBu$_3$P, THF.

but PDE10A inhibitory activity remained less potent than that of MP-10. In addition, compound **1** was found to have a potent inhibition of human cytochrome P450 3A4 (CYP3A4). As CYP3A4 is considered to be involved in the metabolism of more than 50% of drugs approved for human use,[8] inhibition of CYP3A4 is a significant concern regarding drug–drug interactions in clinical practice. Unencumbered basic nitrogens of heterocycles, such as 4-pyridyl ring, bind to the heme portion of CYP enzymes and result in CYP inhibition.[9] The attenuation of CYP3A4 inhibitory activity was therefore assumed to be achieved by replacing pyridine ring or introducing substituents into the pyridine ring of **1**.

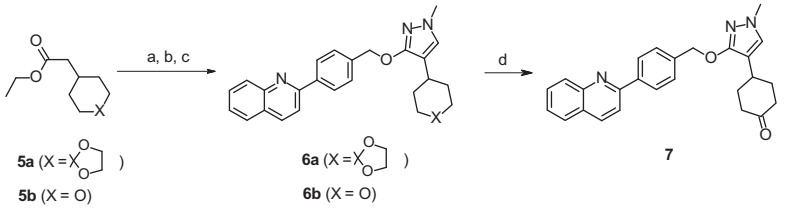
In this paper, we synthesized analogues of our lead compound **1** and discuss SAR of PDE10A inhibitory activity and CYP3A4 inhibition. We also describe the successful development of potent PDE10A inhibitor with reduced CYP3A4 inhibition.

2. Chemistry

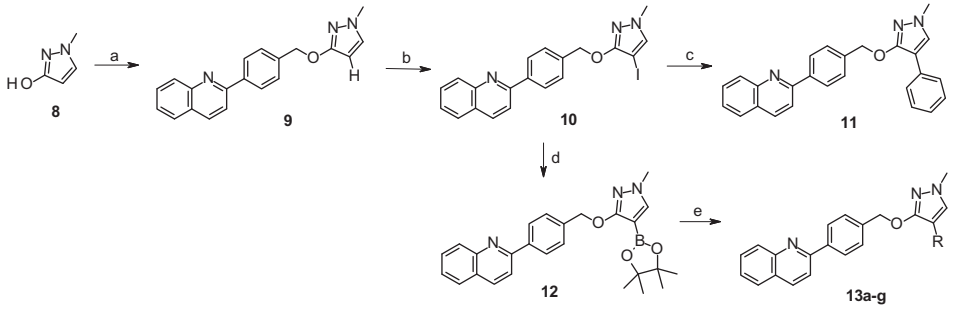
The synthesis of target compounds is shown in Schemes 1–8. The reaction of reagents **2a** and **2b** with *N*,*N*-dimethylformamide dimethyl acetal followed by cyclization with methylhydrazine gave **3a** and **3b**, which were reacted with [4-(quinolin-2-yl)phenyl]methanol under Mitsunobu-type reaction condition to give **4a** and **4b**, respectively. As shown in Scheme 2, compounds **6a** and **6b** were also synthesized in a similar manner from their precursors **5a** and **5b**, respectively. The acetal group of **6a** was deprotected under acidic condition to afford ketone **7**.

Scheme 3 shows the synthesis of **11** and **13a–g**. The Mitsunobu-type reaction between pyrazole analogue **8** and [4-(quinolin-2-yl)phenyl]methanol generated **9**, which was iodinated to give intermediate **10**. The Suzuki coupling reaction between **10** and phenylboronic acid afforded **11**. Halogen–magnesium exchange of intermediate **10** followed by the addition of borate gave the correspondent boronic acid ester **12**,[10] which was then reacted with various substituted halides using Suzuki coupling reaction to yield **13a–g**.

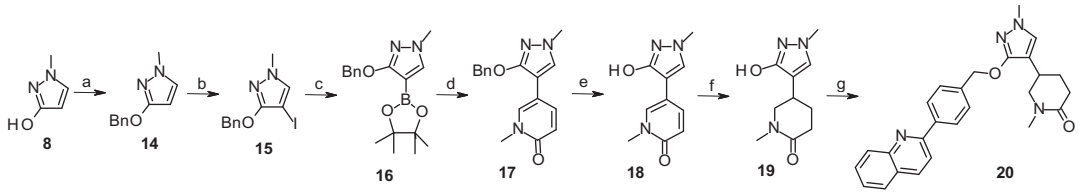
The synthesis of compound **20** is shown in Scheme 4. Pyrazole analogue **8** was alkylated with benzyl bromide to give **14**, which was converted to pyrazole pinacol borate **16** in a manner similar to that of **12**. The Suzuki coupling reaction between **16** and 5-bromo-1-methylpyridone followed by the deprotection of the benzyl group (Bn) yielded intermediate **18**. The pyridone ring of



Scheme 2. Reagents and conditions: (a) LiHMDS, HCO_2Me, THF; (b) methyl hydrazine, EtOH; (c) 2-[4-(chloromethyl)phenyl]quinoline hydrochloride, K_2CO_3, DMF; (d) p-TsOH·H_2O, THF, H_2O.



Scheme 3. Reagents and conditions: (a) [4-(quinolin-2-yl)phenyl]methanol, ADDP, nBu$_3$P, THF; (b) CAN, I_2, MeCN; (c) PhB(OH)$_2$, Pd$_2$(dba)$_3$, Xphos, K_3PO_4, nBuOH; (d) 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, iPrMgCl, THF; (e) R-Br or R-Cl, $PdCl_2$(dppf)·CH_2Cl_2, Na_2CO_3, DMF, H_2O.



Scheme 4. Reagents and conditions: (a) benzyl bromide, K_2CO_3, DMF; (b) CAN, I_2, MeCN; (c) $PdCl_2$(dppf)·CH_2Cl_2, iPrMgCl, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, THF; (d) 5-bromo-1-methylpyridin-2(1*H*)-one, $PdCl_2$(dppf)·CH_2Cl_2, Na_2CO_3, DMF, H_2O; (e) H_2, Pd–C, EtOH; (f) H_2, PtO_2, AcOH; (g) 2-[4-(chloromethyl)phenyl]quinoline hydrochloride, K_2CO_3, DMF.

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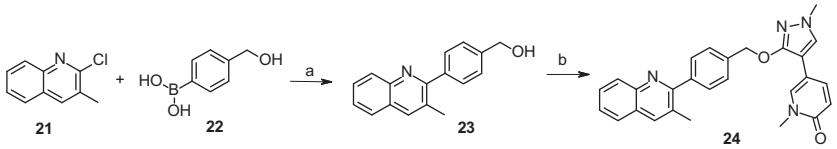
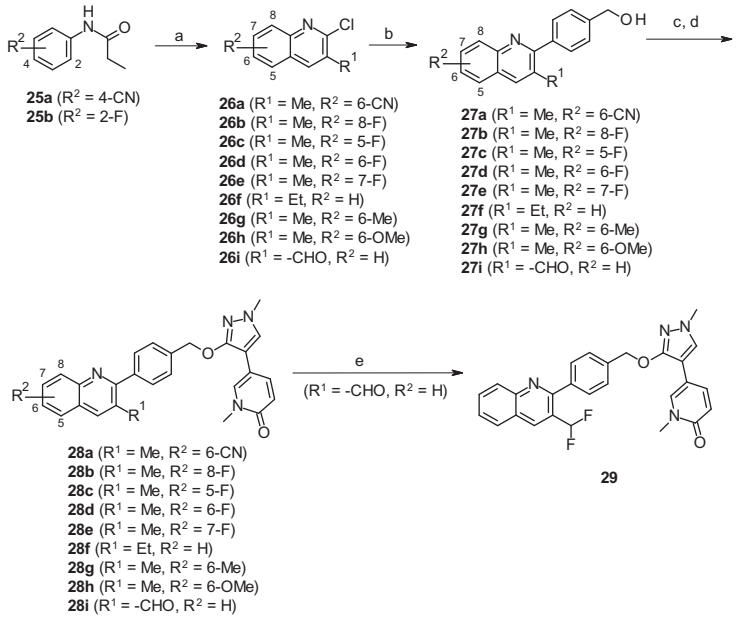
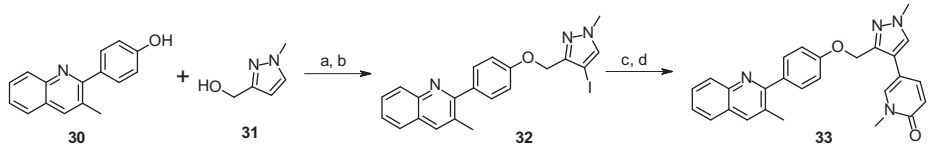


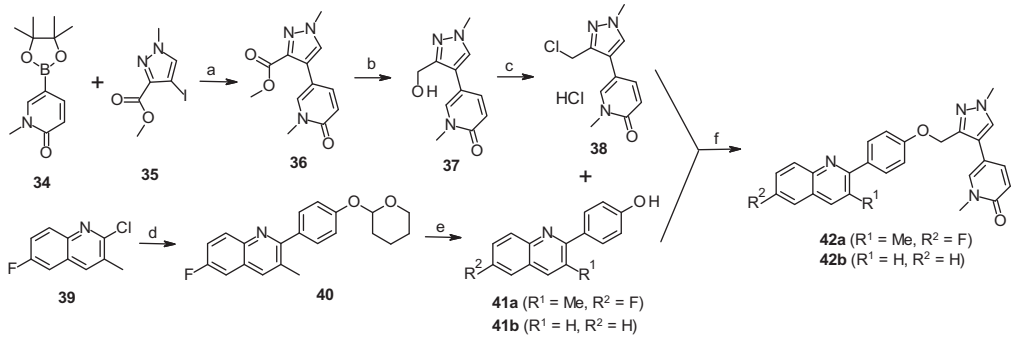
Scheme 5. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O; (b) **18**, CMBP, toluene.



Scheme 6. Reagents and conditions: (a) $POCl_3$, CTAB, DMF; (b) **22**, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O; (c) $SOCl_2$, CH_2Cl_2; (d) **18**, K_2CO_3, DMF; (e) Deoxo-Fluor,® CH_2Cl_2.



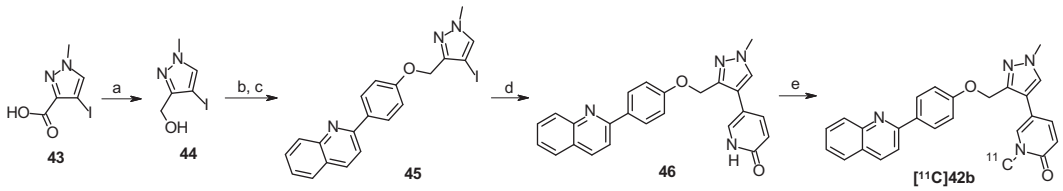
Scheme 7. Reagents and conditions: (a) CMBP, toluene; (b) CAN, I_2, MeCN; (c) isopropoxyboronic acid pinacol ester, *i*PrMgCl, THF; (d) 5-bromo-1-methylpyridin-2(1*H*)-one, $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3, DMF, H_2O.



Scheme 8. Reagents and conditions: (a) $Pd(PPh_3)_4$, Cs_2CO_3, DMF, H_2O; (b) $LiBH_4$, EtOH, THF; (c) $SOCl_2$; (d) 4-(2-tetrahydropyranyloxy)phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O; (e) HCl, THF, H_2O; (f) K_2CO_3, DMF.

intermediate **18** was hydrogenated to give **19**, which was alkylated with 2-[4-(chloromethyl)phenyl]quinoline to afford **20**.

Substituted quinoline derivatives **24**, **28a**–**h**, and **29** were prepared using the synthesis depicted in Schemes 5 and 6. Chloroquinoline analogue **21** and boronic acid **22** underwent Suzuki coupling reaction to give **23**, which was then reacted with compound **18** to give **24**. 2-Chloroquinoline analogues **26a** and **26b** were prepared from **25a** or **25b** via cyclization reaction under Vilsmeier–Haack conditions.[11] Compounds **26d**–**i** were commercially available and the synthesis of **26c** was as previously reported.[12]



Scheme 9. Reagents and conditions: (a) CDI, THF, then $NaBH_4$, H_2O; (b) $SOCl_2$, CH_2Cl_2; (c) **41b**, K_2CO_3, DMF; (d) 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-2-ol, $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3, MeCN, H_2O; (e) [^{11}C]CH_3OTf, NaH, THF, DMSO.

Suzuki coupling reaction between **26a–i** and **22** yielded **27a–i**. The chlorination of hydroxyl group of **27a–i** followed by the reaction with **18** gave **28a–i**. The aldehyde moiety of **28i** was converted to difluoromethyl group using Deoxo-Fluor® to give **29**.

Compound **33** was prepared in four steps as outlined in Scheme 7. Compounds **30** and commercially available alcohol **31** were reacted under Mitsunobu-type reaction condition,[13] followed by iodination of the pyrazole ring to give **32**. Compound **32** was then converted to pyrazole pinacol borate and underwent Suzuki coupling with 5-bromo-1-methylpyridone to afford **33**.

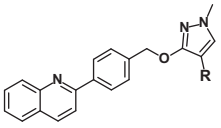
Scheme 8 shows the synthesis of **42a** and **42b**. Suzuki coupling of compound **34** with **35** followed by the reduction of ester group with lithium borohydride gave alcohol **37**. Treatment of **37** with thionyl chloride followed by the etherification with **41a** or **41b** gave desired products **42a** and **42b**, respectively. Compound **41a** was synthesized from its precursor **39** via Suzuki coupling and subsequent deprotection of tetrahydropyranyl group, and the synthesis of **41b** was already reported.[14]

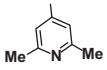
The synthesis of ^{11}C-labeled **42b** ([^{11}C]**42b**) is shown in Scheme 9. The reduction of carboxylic acid of **43** yielded alcohol **44**. Hydroxyl group of **44** was chlorinated with thionyl chloride followed by etherification to give **45**, which was converted to **46** via Suzuki coupling. [^{11}C]**42b** was synthesized from **46** and ^{11}C-labeled methyl trifluoromethanesulfonate, and total synthesis time was 36 min from the end of bombardment (EOB). The decay corrected radiochemical yield was 24–27%, calculated from the end of synthesis, available as: 3.0–3.8 GBq; 95% radiochemically pure and 29–60 GBq/μmol.

3. Results and discussion

PDE10A inhibitory potencies of synthesized compounds were tested using in vitro inhibition of human recombinant PDE10A catalyzed cAMP hydrolysis, and CYP3A4 inhibitory activity was described as the residual activity of midazolam following 30 min of pre-incubation in the presence of compound (5 μM). As shown in Table 1, we first investigated the position of pyridyl nitrogen atom of our lead compound **1**. The rank order of PDE10A inhibitory activity was 4-pyridyl > 3-pyridyl > 2-pyridyl (**1**, **4a**, and **4b**). Regarding PDE10A inhibitory activities, the introduction of methyl group into the pyridine ring of **1** retained activity (**13a**), whereas one more methyl group led to decreased activity (**13b**). As for CYP3A4 inhibition, slight improvement was observed depending on the introduction of methyl group, which indicated that bulkiness of the methyl group hampered the binding of pyridyl nitrogen atom to the heme portion of CYP3A4 enzyme.[9] Substituting the methyl group of **13a** for a methoxy group resulted in a loss of PDE10A inhibitory activity (**13c**). We next examined compound with a phenyl ring at 4-position of the pyrazole ring to confirm the effect on CYP3A4 inhibition. Although **11** totally lost PDE10A inhibitory activity, CYP3A4 inhibition was dramatically improved, indicating that basicity of pyridyl nitrogen atom of **1** contributes to CYP3A4 inhibition and that loss of pyridyl nitrogen atom

Table 1
PDE10A potency and CYP3A4 inhibition



Compd	R	PDE10A IC_{50} (nM)	CYP3A4 residual activity[b] (%)
1		29	19
4a		274	7.5
4b		>1000	NT[a]
13a		25	31
13b		54	48
13c		135	NT[a]
11		>100,000	96
13e		24	59
13d		151	65
13f		73	46
13g		289	78
20		27	49
7		138	NT[a]
6b		155	92

[a] Not tested.
[b] Residual activities of HLM for metabolism of midazolam in the presence of test compounds were determined following preincubation for 30 min. See Section 5.

resulted in the loss of PDE10A inhibitory activity. We hypothesized that a non-basic ring structure with high hydrogen bond acceptor (HBA) ability can support both potent PDE10A inhibitory activity and improved CYP3A4 inhibition. The HBA strength was quantified by calculating the hydrogen bond basicity (pK_{BHX}), and the non-basic *N*-methyl pyridone ring was reported to have high pK_{BHX} value (pK_{BHX} = 2.50).[15] We therefore replaced pyridine ring of **1** with *N*-methyl pyridone ring. Compound **13e** showed similar PDE10A inhibitory activity and improved CYP3A4 inhibition compared with **1**, whereas **13d** showed reduced CYP3A4 inhibition but less PDE10A inhibitory activity, suggesting that a position of the carbonyl group as an HBA is important for PDE10A inhibitory activity. The location of HBA on the extension line of a bond from 4-positon of the pyrazole ring might be important. Although the distance between pyrazole ring and HBA of **13e** is marginally longer than that of **1**, we consider pyridonyl oxygen atom of **13e** and pyridyl nitrogen atom of **1** to be located at similar position inside PDE10A catalytic pocket. Additionally, pyridonyl oxygen atom of **13e** might form hydrogen bond with water molecule inside PDE10A catalytic pocket, as with MP-10.[6e] We further investigated non-basic rings which could function as HBA. The introduction of one more nitrogen atom into pyridone ring of **13e** was detrimental to in vitro activity (**13f**, **13g**). Replacement of pyridone ring of **13e** with saturated piperidone ring retained in vitro activity, but CYP3A4 inhibition of **20** was a little more potent than that of **13e**, which may be due to the higher lipophilicity of **20** (ACD/log *P* = 4.9) compared with **13e** (ACD/log *P* = 3.2).[9,16] Compounds with cyclohexanone or tetrahydropyran ring showed weaker PDE10A inhibitory activity than **13e** (**7**, **6b**). We conducted correlation analyses based on the pIC_{50} values of PDE10A inhibitory activity and the reported pK_{BHX} values listed in Table 2.[15,17] Scatter plot

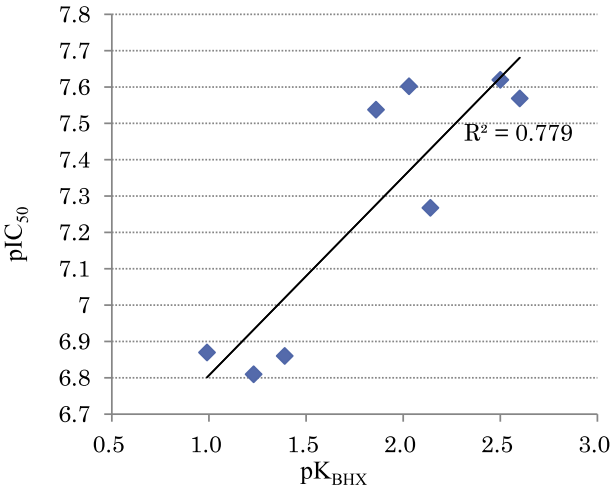
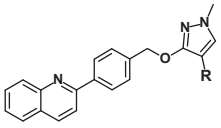


Figure 2. Scatter plot of pIC_{50} against pK_{BHX} values.

Table 2
pIC_{50} of PDE10A inhibitory activity and pK_{BHX} values



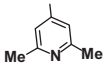
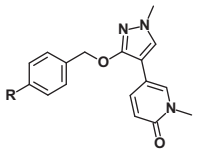
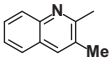
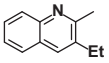
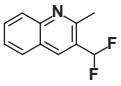
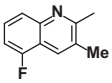
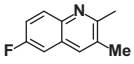
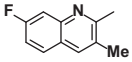
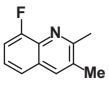
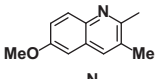
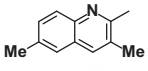
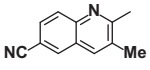
Compd	R	pIC_{50}	pK_{BHX}
1		7.54	1.86
13a		7.60	2.03
13b		7.27	2.14
13c		6.87	0.99
13e		7.59	2.50
20		7.57	2.60
7		6.86	1.39
6b		6.81	1.23

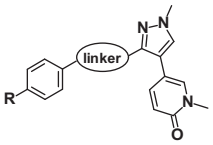
Table 3
Substituent effect on PDE10A potency and CYP3A4 inhibition

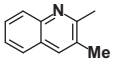
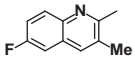
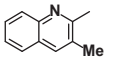
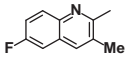


Compd	R	PDE10A IC_{50} (nM)	CYP3A4 residual activity[c] (%)
13e		24	59
24		1.6	30
28f		22	Insoluble[b]
29		100	86
28c		43	53
28d		15	66
28e		52	27
28b		37	35
28h		21	Insoluble[b]
28g		157	70
28a		91	NT[a]

[a] Not tested.
[b] Insoluble in assay buffer.
[c] Residual activities of HLM for metabolism of midazolam in the presence of test compounds were determined following preincubation for 30 min. See Section 5.

Table 4
Effect of linker unit on PDE10A potency, CYP3A4 inhibition and intrinsic clearance



Compd	R	Linker	PDE10A IC_{50} (nM)	CYP3A4 residual activity[a] (%)	h CLint (mL/min/kg)	ACD/log*P*
13e		$-CH_2O-$	24	59	168	3.2
24		$-CH_2O-$	1.6	30	239	3.7
28d		$-CH_2O-$	15	66	263	4.4
42b		$-OCH_2-$	5.1	83	120	2.0
33		$-OCH_2-$	1.6	67	576	2.4
42a		$-OCH_2-$	2.8	82	445	3.2

[a] Residual activities of HLM for metabolism of midazolam in the presence of test compounds were determined following preincubation for 30 min. See Section 5.

of pIC_{50} against pK_{BHX} is shown in Figure 2, and the coefficient of determination showed that the value of R^2 was 0.779, which would explain the positive correlation between the pIC_{50} and the pK_{BHX} values. This correlation analysis result suggested that strength of hydrogen bond between compound and the water molecule inside PDE10A catalytic pocket is important for potent PDE10A inhibitory activity.

We next investigated the substituent effect on quinoline ring of **13e** (Table 3). The introduction of methyl group at 3-position of quinoline ring increased activity (**24**), while the replacement of methyl group of **24** with ethyl group led to decreased PDE10A inhibitory activity (**28f**). Electron donation by methyl group of **24** would increase the HBA ability of quinolynyl nitrogen atom, which may strengthen hydrogen bond between this nitrogen atom and Tyr693 of PDE10A enzyme, improving in vitro activity. When CYP3A4 inhibition of **24** was compared to that of **13e**, methyl group at 3-position of quinoline ring was found to have a negative impact. The ACD/log *P* value of **24** and **13e** was 3.7 and 3.2, respectively, and higher lipophilicity of **24** might lead to high CYP3A4 inhibition. An electron withdrawing difluoromethyl group improved CYP3A4 inhibition but decreased PDE10A inhibitory activity (**29**). We further examined the substituents effect at other positions on the quinoline ring. The introduction of fluorine group reportedly can improve CYP3A4 inhibition,[18] we therefore introduced fluorine atom into quinoline ring of **24**. The fluorine-introduced analogues **28c**, **28d**, **28e** and **28b** showed moderate in vitro activity, with fluorine group at 6-position being optimal (**28d**). The fluorine group at 6-position also gave drastically improved CYP3A4 inhibition (**28d**). We next replaced fluorine group of **28d** with methoxy, methyl or cyano groups. While **28g** with a methyl group and **28a** with a cyano group at the 6-position showed weak activity, **28h** with methoxy group had similar PDE10A inhibitory activity to **28d**. Unfortunately, CYP3A4 inhibition of **28h** was not determined due to its poor solubility in the assay buffer.

As compounds **13e**, **24**, and **28d** had more potent PDE10A inhibitory activity and reduced CYP3A4 inhibition than our lead compound **1**, we finally modified 'oxy-methyl' unit of these compounds. As described above, lower ACD/log *P* value of our quinoline analogues may result in less CYP3A4 inhibition. We therefore conducted a replacement of 'oxy-methyl' unit of **13e**, **24**, and **28d** with 'methyl-oxy' unit, which reduced ACD/log *P* values, as shown in Table 4. As expected, this conversion resulted in a further improvement of CYP3A4 inhibition in all cases (**42b**, **33**, **42a**). Moreover, this conversion had positive impact on PDE10A inhibitory activity. Among compounds listed in Table 4, compound **33** showed the most potent in vitro activity but showed high intrinsic clearance in HLM. Compounds **24** and **42a** also exhibited very potent PDE10A inhibitory activity but still had higher intrinsic clearance in HLM than **13e** or **42b**. These results indicated that a methyl group at 3-position of quinoline ring increased PDE10A inhibitory activity but made compounds metabolically unstable. The X-ray co-crystal structure of **42b** in the catalytic domain of PDE10A enzyme confirmed that the quinolynyl nitrogen atom formed a hydrogen bond to the Tyr693 of PDE10A enzyme, while the quinoline ring forms CH–π interaction with Gly725 and Met713 (Fig. 3). Although water molecules were not assigned in the cocrystal structure of **42b** due to low resolution, pyridonyl oxygen atom of **42b** is located at

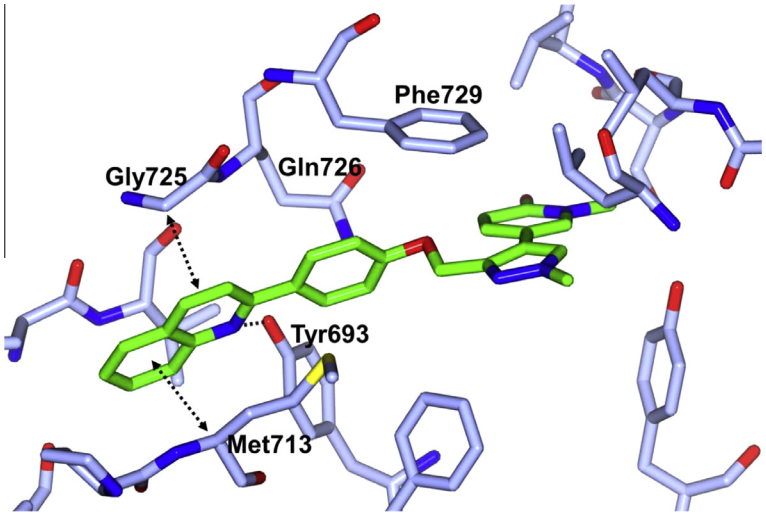


Figure 3. Crystal structure of **42b** (lime green) bound to the PDE10A (PDB code: 4WN1).

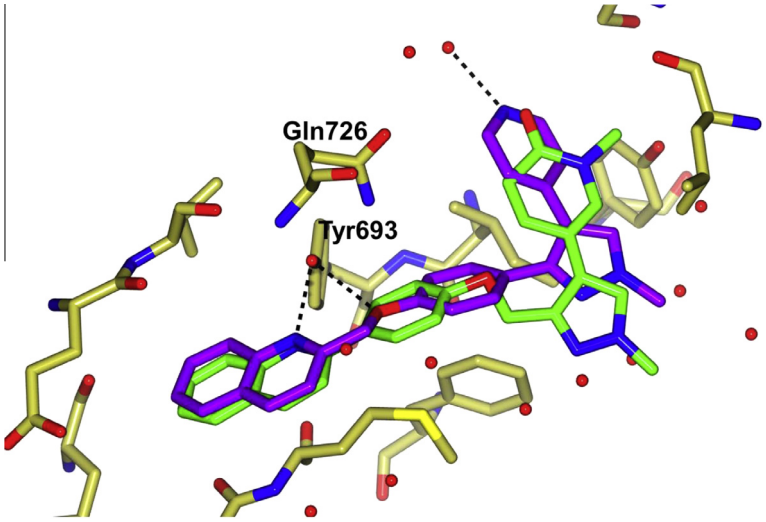


Figure 4. Superimposition of **42b** (green) on the crystal structure of MP-10 (purple) bound PDE10A (PDB code: 3HR1). Red spheres indicate water molecules and dashed lines indicate hydrogen bonds.

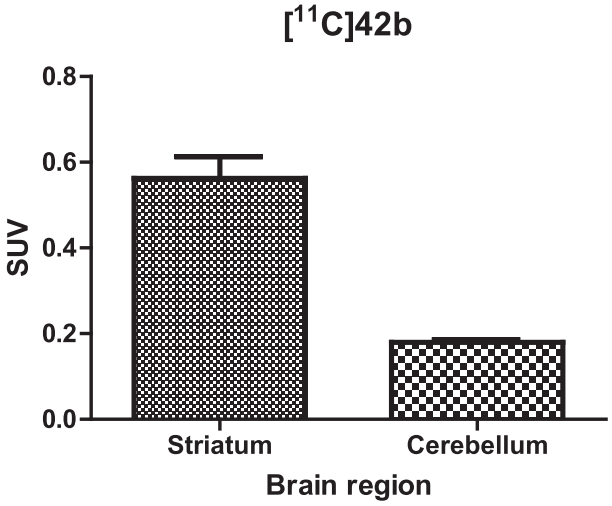


Figure 5. The standardized uptake value (SUV) after intravenous injection of [^{11}C]**42b** in mice striatum and cerebellum.

nearly the same position as pyridyl nitrogen atom of MP-10 and likely binds to the water molecule (Fig. 4).[6e] Figure 4 indicated that pyrazole ring of **42b** is located at a different position from that of MP-10 to assign pyridonyl oxygen atom to a suitable position for possible hydrogen bond to the water molecule.

Compound **42b** exhibited approximately 6-fold more potent activity than **1** and reduced CYP3A4 inhibition and improved metabolic stability in HLM. PDE selectivity of **42b** was also investigated, with results showing a good profile of greater than 1200-fold selectivity over PDE1, 3, 4, 5, 6, 7, 8, 9, and 11. The accumulation of **42b** in striatum using positron-labeled [^{11}C]**42b** was next investigated. In a distribution study in mice, radioactivity in the striatum and cerebellum was measured at 60 min after intravenous injection of [^{11}C]**42b**, which resulted in the higher standardized uptake value (SUV) in striatum than that in cerebellum (Fig. 5). As shown in Figure 6, PET imaging study in rat also showed that [^{11}C]**42b** specifically accumulated in striatum, and blocking with MP-10 (10 mg/kg, iv) resulted in the inhibition of this accumulation. These results indicate that **42b** exhibited good brain penetration and specifically bound to PDE10A in the striatum.

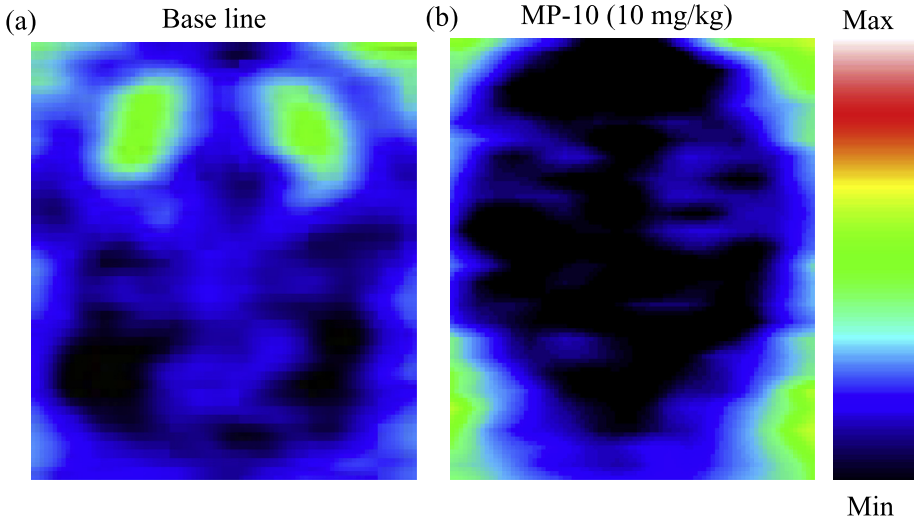


Figure 6. PET images of [^{11}C]**42b** in rat brain. (a) PET image after intravenous administration of [^{11}C]**42b**. (b) PET image after intravenous administration of [^{11}C]**42b** in the presence of MP-10.

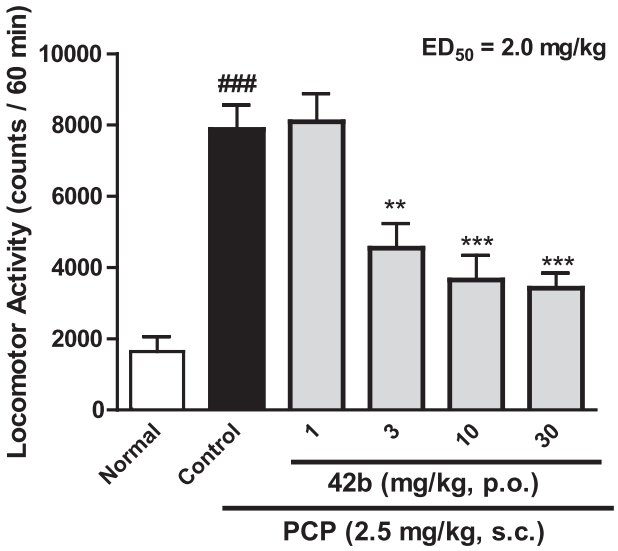


Figure 7. Effect of oral administration of **42b** on PCP-induced hyperlocomotion in mice. PCP was administered subcutaneously (sc). The data represent the mean ± SEM ($n = 8$ in each group): $^{###}p < 0.001$ versus normal group (Student's t test); $^{**}p < 0.01$, $^{***}p < 0.001$ versus control group (Dunnett's test).

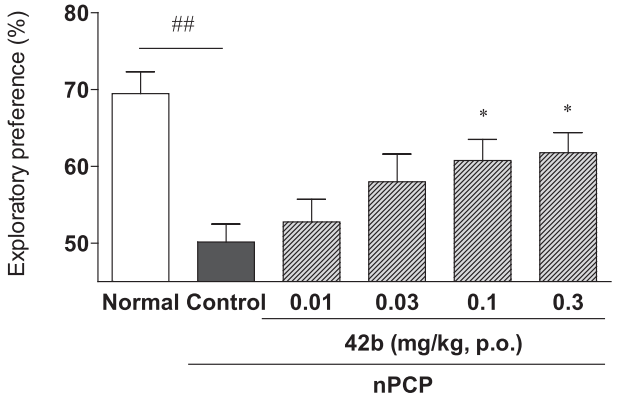


Figure 8. Effect of **42b** on neonatal PCP treatment-induced learning deficit in mice during novel object recognition test. The data represent the mean ± SEM ($n = 13$ or 14 in each group): $^{##}p < 0.01$ versus normal group (Student's t-test); $^{*}p < 0.05$ versus control group (Dunnett's test).

Table 5
Profiles of MP-10 and **42b**

	MP-10	42b
PDE10A IC_{50} (nM)	0.55	5.1
PCP-induced hyperlocomotion in mouse: ED_{50} (mg/kg)	2.5	2.0
CYP3A4 inhibition: residual activity[a] (%)	35	83
CLint (mL/min/kg) (human, mouse)	186, >1000	120, 285

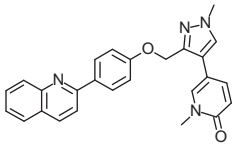
[a] Residual activities of HLM for metabolism of midazolam in the presence of test compounds were determined following preincubation for 30 min. See Section 5.

We also assessed the in vivo behavioral effect on hyperlocomotion induced by phencyclidine (PCP) in mice, an animal model for positive symptom of schizophrenia. As shown in Figure 7, **42b** dose-dependently attenuated the locomotor activity after oral administration, with an ED_{50} value of 2.0 mg/kg. Compound **42b** also significantly improved visual-recognition memory impairment in neonatal PCP-treated mice by the oral administration at 0.1 and 0.3 mg/kg in novel object recognition test (Fig. 8).

Table 6
Brain concentration of MP-10 and **42b** after 3.0 mg/kg oral dosage

Compd	Brain concd (ng/mg)	Fu, brain[a]	Free brain concd[b] (ng/mg)
MP-10	99	0.003	0.30
42b	163	0.006	1.0

[a] Unbound fraction in brain tissue.
[b] Free brain concd was calculated by multiplying brain concd by fu,brain.



42b

in vitro properties

PDE10A IC_{50}: 5.1 nM
other PDEs: >1200 fold

in vivo properties

mice PCP-HL ED_{50}: 2.0 mg/kg, po
mice NORT MED: 0.1 mg/kg, po

Pharmacokinetics

CYP inhibition (1A2, 2C9, 2C19, 2D6); IC_{50}: >10uM
CYP3A4 inhibition; residual activity: 83%
CLint (mL/min/kg) (human, mouse, rat, dog, monkey): 120, 285, 484, 148, 299
F (1.0 mg/kg; rat, dog, monkey): 45, 112, 105 %
CL_{total} (mL/min/kg) (i.v.; rat, dog, monkey): 12, 1.1, 1.7 (mL/min/kg)
$t_{1/2}$ (h) (i.v.; rat, dog, monkey) : 1.3, 4.5, 2.5

Figure 9. Profiles of compound **42b**.

Profiles of **42b** and MP-10 were shown in Table 5. Compound **42b** was more stable in human and mouse liver microsomes than MP-10, and **42b** showed less CYP3A4 inhibition over MP-10. The in vitro PDE10A inhibitory activity of **42b** was 9-fold lower than that of MP-10, but in vivo potency in PCP-induced hyperlocomotion model was nearly equal between the two compounds. Brain free concentrations of **42b** and MP-10 at 1 h after oral administration of 3.0 mg/kg to mice were calculated as 1.0 ng/g and 0.3 ng/g, respectively (Table 6). The higher free concentration of **42b** than MP-10 would contribute to its in vivo potency. Additional profiles of **42b** are shown in Figure 9. Compound **42b** exhibited low CYP1A2, 2C9, 2C19, and 2D6 inhibition in addition to its low CYP3A4 inhibition. Compound **42b** was adequately stable in rat, dog, and monkey liver microsomes, and showed good PK profiles in rat, dog, and monkey. These results indicated that **42b** would have a potential to be used for the treatment of schizophrenia with less concern for drug–drug interactions compared to MP-10.

4. Conclusions

In this study, we investigated the SAR of PDE10A inhibitory activity and CYP3A4 inhibition to obtain potent PDE10A inhibitors with less concern for drug–drug interactions. Substituting pyridine ring of **1** with non-basic pyridone ring gave **13e** with improved CYP3A4 inhibition, and the positive correlation was observed between the pIC_{50} of PDE10A inhibitory activity and the pK_{BHX} values of substituent at 4-position of pyrazole ring. Replacement of 'oxy-methyl' unit of **13e** with 'methyl-oxy' unit to give **42b** resulted in improved PDE10A inhibitory activity and further reduced CYP3A4 inhibition. PET study indicated that **42b** exhibited good brain penetration and specifically accumulated in rodent striatum. Further, compound **42b** attenuated the locomotor activity induced by PCP after oral administration with an ED_{50} value of 2.0 mg/kg, and **42b** significantly improved visual-recognition memory impairment by the oral administration at 0.1 and 0.3 mg/kg in novel object recognition test.

5. Experimental section

5.1. Chemistry

^{1}H NMR spectra were recorded on a Varian VNS-400, JEOL JNM-LA400, or JEOL JNM-AL400 and the chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal reference (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, and br = broad peak). Mass spectra (MS) were recorded on Thermo Electron LCQ Advantage or Agilent 6140. Elemental analyses were performed using Yanaco MT-6 (C, H, N), Elementar Vario EL III (C, H, X), and Dionex ICS-3000 (S, halogene) and were within ±0.4% of theoretical values.

5.1.1. 1-Methyl-4-(pyridin-3-yl)-1*H*-pyrazol-3-ol (3a)

A mixture of ethyl pyridin-3-ylacetate (**2a**, 2.00 g, 12.1 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (3.61 g, 30.3 mmol) in DMF (10 mL) was stirred at 110 °C for 2 h. After cooling at room temperature, the mixture was concentrated in vacuo to give a reddish brown oil. To this reddish brown oil in EtOH (20 mL) were added acetic acid (5.0 mL) and methylhydrazine (1.12 g, 24.2 mmol), and the mixture was stirred at room temperature for 16 h before the mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (0–10% MeOH in $CHCl_3$) to give **3a** (620 mg, 29%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 3.67 (s, 3H), 7.29–7.34 (m, 1H), 7.94–7.99 (m, 2H), 8.30 (dd, 1H, J = 4.7, 1.6 Hz), 8.83 (d, 1H, J = 1.6 Hz), 10.43 (br s, 1H); MS (ESI) m/z 176 $[M+H]^+$.

5.1.2. 1-Methyl-4-(pyridin-2-yl)-1*H*-pyrazol-3-ol (3b)

Compound **3b** was prepared from **2b** in a manner similar to that described for compound **3a**, with a yield of 45% as a brown solid. ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3H), 7.10–7.15 (m, 1H), 7.65–7.69 (m, 1H), 7.75–7.80 (m, 1H), 8.07 (s, 1H), 8.44–8.46 (m, 1H), 10.94 (br s, 1H); MS (ESI) m/z 176 $[M+H]^+$.

5.1.3. 2-[4-({[1-Methyl-4-(pyridin-3-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline dihydrochloride (4a)

To a stirred mixture of **3a** (263 mg, 1.50 mmol) and [4-(quinolin-2-yl)phenyl]methanol (388 mg, 1.65 mmol) in THF (20 mL) were added 1,1′-(azodicarbonyl)-dipiperidine (ADDP, 568 mg, 2.25 mmol) and tributylphosphine (455 mg, 2.25 mmol), and the mixture was stirred at room temperature for 12 h before the mixture was concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; 0–5% MeOH in $CHCl_3$, then NH silica gel; 20–50% EtOAc in hexane) to give a free form of the title compound, which was diluted with EtOH (20 mL) and treated with 4 M HCl/EtOAc (1.5 mL). After the mixture was stirred at room temperature for 30 min, the mixture was concentrated in vacuo and recrystallized from EtOH/EtOAc to give **4a** (446 mg, 64%) as a cream-colored solid. ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 3H), 5.49 (s, 2H), 7.66–7.77 (m, 3H), 7.85–7.93 (m, 1H), 8.05 (dd, 1H, J = 8.2, 5.7 Hz), 8.11 (d, 1H, J = 7.9 Hz), 8.25 (d, 2H, J = 8.7 Hz), 8.33 (d, 2H, J = 8.4 Hz), 8.45 (s, 1H), 8.62–8.70 (m, 3H), 8.71–8.75 (m, 1H); MS (ESI) m/z 393 $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_4O\cdot 2HCl\cdot 3.3H_2O$: C, 57.21; H, 5.49; N, 10.68; Cl, 13.51. Found: C, 57.09; H, 5.77; N, 10.31; Cl, 13.89.

5.1.4. 2-[4-({[1-Methyl-4-(pyridin-2-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline dihydrochloride (4b)

Compound **4b** was prepared from **3b** in a manner similar to that described for compound **4a**, with a yield of 72% as a cream-colored solid. ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 5.53 (s, 2H), 7.67–7.78 (m, 4H), 7.89–7.95 (m, 1H), 8.14 (d, 1H, J = 7.9 Hz), 8.23 (d, 1H, J = 8.3 Hz), 8.26–8.36 (m, 4H), 8.44–8.50 (m, 1H), 8.64–8.67 (m, 1H), 8.72 (d, 1H, J = 8.6 Hz), 8.88 (s, 1H); MS (ESI) m/z 393 $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_4O\cdot 2HCl\cdot 0.4C_2H_6O\cdot 1.9H_2O$: C, 59.82; H, 5.49; N, 10.82; Cl, 13.69. Found: C, 59.51; H, 5.81; N, 10.42; Cl, 14.06.

5.1.5. 2-[4-({[4-(1,4-Dioxaspiro[4.5]dec-8-yl)-1-methyl-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline (6a)

To a stirred mixture of lithium bis(trimethylsilyl)amide (1 M solution in THF, 30.1 mL, 30.1 mmol) in THF (45 mL) cooled with dry ice–acetone bath were dropwisely added a solution of ethyl 1,4-dioxaspiro[4.5]dec-8-ylacetate (**5a**, 6.55 g, 28.7 mmol) with keeping the temperature below −65 °C, and the mixture was stirred at the same temperature for 50 min. To the resultant mixture was dropwisely added methyl formate (3.45 g, 57.4 mmol) with keeping the temperature below −65 °C, and the mixture was stirred at the same temperature for 10 min. The resultant mixture was allowed to warm up to room temperature and stirred for 3 h. The reaction was cooled with ice-water bath and quenched with ca. 40 mL of 1 M HCl aqueous solution. The mixture was diluted with brine and extracted with $CHCl_3$, The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo to give an orange oil. To this orange oil in EtOH (59 mL) was added methylhydrazine (2.64 g, 57.4 mmol), and the mixture was stirred at 100 °C for 3 h before cooling at room temperature. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (0–15% MeOH in $CHCl_3$) to give a white solid (2.91 g). To this white solid (1.48 g) and 2-[4-(chloromethyl)phenyl]quinoline hydrochloride (1.8 g, 6.20 mmol) in DMF (18 mL) was added K_2CO_3 (2.14 g, 15.5 mmol), and the mixture was stirred at 60 °C for 1 h before cooling at room temperature. The mixture was diluted with water and brine, and the mixture was extracted with $CHCl_3$/MeOH (5:1) for 3 times. The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–3% MeOH in $CHCl_3$) to give **6a** (1.10 g, 17%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.46–1.72 (m, 8H), 2.35–2.44 (m, 1H), 3.56 (s, 3H), 3.83–3.86 (m, 4H), 5.14 (s, 2H), 7.15 (s, 1H), 7.60–7.65 (m, 3H), 7.77–7.83 (m, 1H), 8.02 (d, 1H, J = 8.2 Hz), 8.09 (d, 1H, J = 8.4 Hz), 8.19 (d, 1H, J = 8.7 Hz), 8.33 (d, 2H, J = 8.4 Hz), 8.48 (d, 1H, J = 8.7 Hz); MS (ESI) m/z 456 $[M+H]^+$.

5.1.6. 2-[4-({[1-Methyl-4-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline hydrochloride (6b)

Free form of title compound was prepared from **5b** in a manner similar to that described for compound **6a**, with a yield of 25% as a colorless oil. To this colorless oil (139 mg, 0.35 mmol) in EtOAc (9.7 mL) was added 4 M HCl/EtOAc (0.174 mL), and the mixture was stirred at room temperature for 1 h. The precipitate was collected by filtration to give **6b** (76 mg, 50%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 1.46–1.66 (m, 4H), 2.53–2.63 (m, 1H), 3.33 (td, 2H, J = 11.5, 2.5 Hz), 3.58 (s, 3H), 3.79–3.88 (m, 2H), 5.19 (s, 2H), 7.27 (s, 1H), 7.68 (d, 2H, J = 8.4 Hz), 7.72 (d, 1H, J = 7.9 Hz), 7.86–7.95 (m, 1H), 8.08–8.17 (m, 1H), 8.20–8.39 (m, 4H), 8.69 (d, 1H, J = 8.4 Hz); MS (ESI) m/z 400 $[M+H]^+$; Anal. Calcd for $C_{25}H_{25}N_3O_2\cdot 1.55HCl\cdot 1.2H_2O$: C, 62.87; H, 6.11; N, 8.80; Cl, 11.51. Found: C, 62.96; H, 6.23; N, 8.90; Cl, 11.45.

5.1.7. 4-(1-Methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)cyclohexanone dihydrochloride (7)

To a stirred solution of **6a** (1.10 g, 2.40 mmol) in THF (11 mL) and water (11 mL) was added 4-methylbenzenesulfonic acid hydrate (pTsOH·H_2O, 229 mg, 1.20 mmol), and the mixture was stirred at room temperature for 3 days. The mixture was diluted with $NaHCO_3$ aqueous solution and brine, and extracted with $CHCl_3$ for 2 times. The combined organic layer was dried over

$MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–3% MeOH in $CHCl_3$) to give a free form of the title compound (989 mg, quant) as a pale yellow oil. To this pale yellow oil (117 mg, 0.28 mmol) in EtOAc (3.5 mL) cooled with ice–water bath was added 4 M HCl/EtOAc (0.21 mL, 0.84 mmol), and the mixture was stirred at the same temperature for 15 min. The precipitate was collected by filtration to give **7** (105 mg, 82%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.65–1.79 (m, 2H), 2.09–2.28 (m, 4H), 2.43–2.57 (m, 2H), 2.83–2.93 (m, 1H), 3.32 (s, 3H), 5.15 (s, 2H), 7.45 (d, 2H, J = 8.3 Hz), 7.71 (t, 1H, J = 7.3 Hz), 7.90 (t, 1H, J = 7.3 Hz), 8.00 (s, 1H), 8.12 (t, 1H, J = 8.0 Hz), 8.20–8.29 (m, 4H), 8.69 (s, 1H); MS (ESI) m/z 412 $[M+H]^+$; Anal. Calcd for $C_{26}H_{25}N_3O_2{\cdot}1.7HCl{\cdot}1.75H_2O$: C, 61.95; H, 6.02; N, 8.34; Cl, 11.96. Found: C, 62.10; H, 6.35; N, 8.36; Cl, 12.08.

5.1.8. 2-(4-{[(1-Methyl-1*H*-pyrazol-3-yl)oxy]methyl}phenyl) quinoline (9)

To a solution of 1-methyl-1*H*-pyrazol-3-ol (**8**, 1.01 g, 10.3 mmol), 4-(quinolin-2-yl)phenyl)methanol (2.43 g, 10.3 mmol) and ADDP (3.91 g, 15.5 mmol) in THF (122 mL) was added tributylphosphine (3.14 g, 15.5 mmol), and the mixture was stirred at room temperature for 6 h. The precipitate was filtered off and the filtrate was concentrated in vacuo. The residue was suspended in EtOAc, and the insoluble material was removed by filtration and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–50% EtOAc in hexane) to give **9** (2.20 g, 68%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3H), 5.21 (s, 2H), 5.71 (d, 1H, J = 2.3 Hz), 7.49 (d, 1H, J = 2.3 Hz), 7.58–7.63 (m, 3H), 7.77–7.81 (m, 1H), 8.01 (dd, 1H, J = 8.2, 1.1 Hz), 8.08 (d, 1H, J = 8.2 Hz), 8.16 (d, 1H, J = 8.7 Hz), 8.28–8.31 (m, 2H), 8.47 (d, 1H, J = 8.4 Hz); MS (ESI) m/z 316 $[M+H]^+$.

5.1.9. 2-(4-{[(4-Iodo-1-methyl-1*H*-pyrazol-3-yl)oxy]methyl} phenyl)quinoline (10)

To a solution of **9** (2.20 g, 6.97 mmol) in MeCN (44 mL) were added ceric ammonium nitrate (CAN, 2.29 g, 4.18 mmol) and iodine (1.06 g, 4.18 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated in vacuo, and to the residue were added $CHCl_3$ and 5% $NaHSO_3$ aqueous solution during ice-water cooling. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–30% EtOAc in hexane) to give **10** (893 mg, 29%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H), 5.34 (s, 2H), 7.20 (s, 1H), 7.50–7.55 (m, 1H), 7.62 (d, 2H, J = 8.1 Hz), 7.70–7.75 (m, 1H), 7.83 (d, 1H, J = 8.1 Hz), 7.89 (d, 1H, J = 8.6 Hz), 8.15–8.20 (m, 3H), 8.23 (d, 1H, J = 8.6 Hz); MS (ESI) m/z 442 $[M+H]^+$.

5.1.10. 2-(4-{[(1-Methyl-4-phenyl-1*H*-pyrazol-3-yl)oxy]methyl} phenyl)quinoline hydrochloride (11)

To a mixture of **10** (300 mg, 0.68 mmol), phenylboronic acid (124 mg, 1.02 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (Xphos, 65 mg, 0.14 mmol), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$; 62 mg, 0.068 mmol), and K_3PO_4 (289 mg, 1.36 mmol) was added *n*-butanol (2 mL) under an argon gas atmosphere, and the mixture was stirred at 80 °C for 90 min. The mixture was concentrated in vacuo, and the residue was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–30% EtOAc in hexane) to give a free form of the title compound, which was dissolved in a mixture of EtOH and EtOAc. The solution was treated with 4 M HCl/AcOEt and concentrated in vacuo. The residue was washed with a mixture of EtOH and Et_2O to give **11** (135 mg, 46%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H), 5.41 (s, 2H), 7.13–7.17 (m, 1H), 7.31–7.36 (m, 2H), 7.65–7.71 (m, 5H), 7.83–7.89 (m, 1H), 8.03 (s, 1H), 8.08 (d, 1H, J = 7.8 Hz), 8.16 (d, 1H, J = 8.4 Hz), 8.23 (d, 1H, J = 8.7 Hz), 8.31 (d, 2H, J = 8.4 Hz), 8.61 (d, 1H, J = 8.7 Hz); MS (ESI) m/z 392 $[M+H]^+$; Anal. Calcd for $C_{26}H_{21}N_3O{\cdot}0.6HCl{\cdot}0.6H_2O$: C, 73.62; H, 5.42; N, 9.91; Cl, 5.02. Found: C, 73.81; H, 5.42; N, 10.03; Cl, 4.80.

5.1.11. 2-[4-({[1-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline (12)

To a mixture of **10** (2.0 g, 4.53 mmol) in THF (40 mL) cooled with MeOH–ice bath was dropwisely added isopropylmagnesium chloride (2 M in THF solution, 2.60 mL, 5.20 mmol) with keeping the temperature below −10 °C, and the mixture was stirred at temperature between −18 and 10 °C for 45 min. To the resultant mixture cooled with MeOH–ice bath was added 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.39 mL, 6.80 mmol), and the mixture was allowed to stir at room temperature for 90 min. To the resultant mixture was cooled at −15 °C were added isopropylmagnesium chloride (2 M in THF solution, 1.00 mL, 2.00 mmol) and 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (422 mg, 2.27 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was diluted with EtOAc and washed with saturated NH_4Cl aqueous solution and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–40% EtOAc in hexane) to give **12** as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 12H), 3.73 (s, 3H), 5.40 (s, 2H), 7.45 (s, 1H), 7.49–7.55 (m, 1H), 7.65 (d, 2H, J = 8.4 Hz), 7.70–7.75 (m, 1H), 7.83 (d, 1H, J = 8.1 Hz), 7.89 (d, 1H, J = 8.6 Hz), 8.13–8.18 (m, 3H), 8.22 (d, 1H, J = 8.6 Hz); MS (ESI) m/z 442 $[M+H]^+$.

5.1.12. 2-[4-({[1-Methyl-4-(2-methylpyridin-4-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline dihydrochloride (13a)

Under argon gas atmosphere, to the mixture of **12** (200 mg, 0.45 mmol), 4-bromo-2-methylpyridine (156 mg, 0.91 mmol), and Na_2CO_3 (144 mg, 1.36 mmol) in DMF (2.0 mL) and water (0.87 mL) was added [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane complex ($PdCl_2$(dppf)·CH_2Cl_2, 22 mg, 0.027 mmol), and the mixture was stirred at 100 °C for 1 h. After cooling at room temperature, the mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give a free from of the title compound, which was dissolved in MeOH and the mixture was treated with 4 M HCl/EtOAc. The precipitate formed was collected by filtration and washed with Et_2O to give **13a** (91 mg, 42%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.67 (s, 3H), 3.83 (s, 3H), 5.51 (s, 2H), 7.68 (t, 1H, J = 7.1 Hz), 7.73 (d, 2H, J = 8.4 Hz), 7.84–7.89 (m, 1H), 7.98 (dd, 1H, J = 6.5, 1.8 Hz), 8.03 (br d, 1H, J = 1.8 Hz), 8.08 (d, 1H, J = 7.8 Hz), 8.20 (d, 1H, J = 8.4 Hz), 8.24 (d, 1H, J = 8.7 Hz), 8.34 (d, 2H, J = 8.4 Hz), 8.58–8.65 (m, 3H); MS (ESI) m/z 407 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O{\cdot}2.3HCl{\cdot}3H_2O$: C, 57.36; H, 5.61; N, 10.29; Cl, 14.98. Found: C, 57.28; H, 5.67; N, 10.15; Cl, 15.22.

5.1.13. 2-[4-({[4-(2,6-Dimethylpyridin-4-yl)-1-methyl-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline dihydrochloride (13b)

Compound **13b** was prepared from **12** and 4-bromo-2,6-dimethylpyridine hydrobromide in a manner similar to that described for compound **13a**, with a yield of 62% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.65 (s, 6H), 3.82 (s, 3H), 5.52 (s, 2H), 7.67 (t, 1H, J = 7.5 Hz), 7.72 (d, 2H, J = 8.4 Hz), 7.85–7.88 (m, 3H), 8.08 (d, 1H, J = 7.9 Hz), 8.19 (d, 1H, J = 8.4 Hz), 8.23 (d, 1H, J = 8.7 Hz), 8.34 (d, 2H, J = 8.3 Hz), 8.57 (s, 1H), 8.61 (d, 1H, J = 8.7 Hz); MS (ESI) m/z 421 $[M+H]^+$; Anal. Calcd for $C_{27}H_{24}N_4O{\cdot}2.2HCl{\cdot}3.5H_2O$: C, 57.52; H, 5.94; N, 9.94; Cl, 13.83. Found: C, 57.87; H, 5.83; N, 9.81; Cl, 13.64.

5.1.14. 2-[4-({[4-(2-Methoxypyridin-4-yl)-1-methyl-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline dihydrochloride (13c)

Compound **13c** was prepared from **12** and 4-bromo-2-methoxylpyridine in a manner similar to that described for compound **13a**, with a yield of 51% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H), 3.95 (s, 3H), 5.47 (s, 2H), 7.29 (s, 1H), 7.41 (dd, 1H, J = 5.9, 1.3 Hz), 7.71–7.76 (m, 3H), 7.89–7.95 (m, 1H), 8.13 (d, 1H, J = 8.2 Hz), 8.17 (d, 1H, J = 5.9 Hz), 8.28 (d, 2H, J = 8.7 Hz), 8.33 (d, 2H, J = 8.4 Hz), 8.45 (s, 1H), 8.72 (d, 1H, J = 8.6 Hz); MS (ESI) m/z 423 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O_2{\cdot}2.2HCl{\cdot}3.5H_2O$: C, 56.10; H, 5.58; N, 10.06; Cl, 12.74. Found: C, 56.33; H, 5.68; N, 9.88; Cl, 12.52.

5.1.15. 1-Methyl-4-(1-methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)pyridin-2(1*H*)-one dihydrochloride (13d)

Compound **13d** was prepared from **12** and 4-bromo-1-methylpyridin-2(1*H*)-one in a manner similar to that described for compound **13a**, with a yield of 68% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.39 (s, 3H), 3.75 (s, 3H), 5.42 (s, 2H), 6.54 (dd, 1H, J = 7.1, 2.0 Hz), 6.74 (d, 1H, J = 1.9 Hz), 7.65 (d, 1H, J = 7.1 Hz), 7.68–7.73 (m, 3H), 7.87–7.93 (m, 1H), 8.11 (d, 1H, J = 8.0 Hz), 8.19–8.23 (m, 2H), 8.27 (d, 1H, J = 8.7 Hz), 8.31 (d, 2H, J = 8.4 Hz), 8.68 (br d, 1H, J = 8.7 Hz); MS (ESI) m/z 423 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O_2{\cdot}2HCl{\cdot}4H_2O$: C, 55.03; H, 5.68; N, 9.87; Cl, 12.50. Found: C, 55.15; H, 5.84; N, 9.58; Cl, 12.46.

5.1.16. 1-Methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)pyridin-2(1*H*)-one dihydrochloride (13e)

Compound **13e** was prepared from **12** and 5-bromo-1-methylpyridin-2(1*H*)-one in a manner similar to that described for compound **13a**, with a yield of 39% as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.46 (s, 3H), 3.72 (s, 3H), 5.41 (s, 2H), 6.47 (d, 1H, J = 9.4 Hz), 7.67–7.78 (m, 4H), 7.87 (s, 1H), 7.92–7.97 (m, 2H), 8.16 (d, 1H, J = 7.9 Hz), 8.28–8.34 (m, 4H), 8.77 (d, 1H, J = 8.7 Hz); MS (ESI) m/z 423 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O_2{\cdot}2.2HCl{\cdot}2.7H_2O$: C, 56.64; H, 5.41; N, 10.16; Cl, 14.15. Found: C, 56.97; H, 5.78; N, 9.93; Cl, 14.25.

5.1.17. 1-Methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)pyrimidin-2(1*H*)-one dihydrochloride (13f)

Compound **13f** was prepared from **12** and 5-bromo-1-methylpyrimidin-2(1*H*)-one in a manner similar to that described for compound **13a**, with a yield of 37% as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.55 (s, 3H), 3.75 (s, 3H), 5.40 (s, 2H), 7.64 (m, 3H), 7.83–7.88 (m, 1H), 7.96 (s, 1H), 8.07 (d, 1H, J = 8.1 Hz), 8.16 (d, 1H, J = 8.6 Hz), 8.22 (d, 1H, J = 8.7 Hz), 8.30 (d, 2H, J = 8.4 Hz), 8.60 (d, 1H, J = 8.7 Hz), 8.67 (d, 1H, J = 3.2 Hz), 8.86 (d, 1H, J = 3.2 Hz); MS (ESI) m/z 424 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}N_5O_2{\cdot}2HCl{\cdot}3.3H_2O$: C, 54.02; H, 5.37; N, 12.60; Cl, 12.76. Found: C, 54.03; H, 5.66; N, 12.46; Cl, 12.44.

5.1.18. 2-Methyl-6-(1-methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)pyridazin-3(2*H*)-one hydrochloride (13g)

Compound **13g** was prepared from **12** and 6-chloro-2-methylpyridazin-3(2*H*)-one in a manner similar to that described for compound **13a**, with a yield of 49% as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.67 (s, 3H), 3.76 (s, 3H), 5.43 (s, 2H), 6.98 (d, 1H, J = 9.6 Hz), 7.73–7.81 (m, 4H), 7.95–8.01 (m, 1H), 8.04 (s, 1H), 8.19 (d, 1H, J = 8.0 Hz), 8.30–8.34 (m, 3H), 8.41 (d, 1H, J = 8.5 Hz), 8.85 (d, 1H, J = 8.5 Hz); MS (ESI) m/z 424 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}N_5O_2{\cdot}1.5HCl{\cdot}1.7H_2O$: C, 59.02; H, 5.13; N, 13.76; Cl, 10.45. Found: C, 59.24; H, 5.53; N, 13.79; Cl, 10.33.

5.1.19. [4-(3-Methylquinolin-2-yl)phenyl]methanol (23)

To a suspension of 2-chloro-3-methylquinoline (**21**, 533 mg, 3.00 mmol) and [4-(hydroxymethyl)phenyl]boronic acid (**22**, 501 mg, 3.30 mmol) in 1,2-dimethoxyethane (20 mL) were added $Pd(PPh_3)_4$ (173 mg, 0.15 mmol) and 1 M Na_2CO_3 aqueous solution (7.5 mL), and the mixture was stirred at 90 °C for 19 h under argon gas atmosphere. After cooling at room temperature, the mixture was partitioned between EtOAc and water. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give **23** as a pale yellow oil. ^{1}H NMR (DMSO-d_6) δ 2.46 (s, 3H), 4.60 (d, 2H, J = 5.7 Hz), 5.27 (t, 1H, J = 5.7 Hz), 7.45 (d, 2H, J = 8.3 Hz), 7.56–7.65 (m, 3H), 7.67–7.73 (m, 1H), 7.93 (d, 1H, J = 8.1 Hz), 7.98 (d, 1H, J = 8.2 Hz), 8.25 (s, 1H); MS (ESI) m/z 250 $[M+H]^+$.

5.1.20. 3-(Benzyloxy)-1-methyl-1*H*-pyrazole (14)

To a suspension of **8** (8.09 g, 82.5 mmol) and K_2CO_3 (13.7 g, 99.0 mmol) in DMF (100 mL) cooled with ice–water bath was added benzylbromide (16.9 g, 98.6 mmol), and the mixture was allowed to stir at room temperature for 1.5 h. The mixture was stirred at 50 °C for another 4 h. The resultant mixture was partitioned between EtOAc and water, and the organic layer was washed with NaCl aqueous solution, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (10–30% EtOAc in hexane) to give **14** (13.1 g, 84%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3H), 5.18 (s, 2H), 5.64 (d, 1H, J = 2.3 Hz), 7.12 (d, 1H, J = 2.3 Hz), 7.28–7.33 (m, 1H), 7.34–7.39 (m, 2H), 7.43–7.46 (m, 2H); MS (ESI) m/z 189 $[M+H]^+$.

5.1.21. 3-(Benzyloxy)-4-iodo-1-methyl-1*H*-pyrazole (15)

Compound **15** was prepared from **14** in a manner similar to that described for compound **10** with a yield of 61% as a brown oil. ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H), 5.25 (s, 2H), 7.19 (s, 1H), 7.29–7.33 (m, 1H), 7.35–7.40 (m, 2H), 7.45–7.48 (m, 2H); MS (ESI) m/z 315 $[M+H]^+$.

5.1.22. 3-(Benzyloxy)-1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (16)

Compound **16** was prepared from **15** in a manner similar to that described for compound **12** with a yield of 82% as a gum. ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 12H), 3.72 (s, 3H), 5.32 (s, 2H), 7.24–7.30 (m, 1H), 7.32–7.37 (m, 2H), 7.43 (s, 1H), 7.49 (d, 2H, J = 7.5 Hz); MS (ESI) m/z 315 $[M+H]^+$.

5.1.23. 5-[3-(Benzyloxy)-1-methyl-1*H*-pyrazol-4-yl]-1-methylpyridin-2(1*H*)-one (17)

Under argon gas atmosphere, to the mixture of **16** (3.5 g, 11.1 mmol), 5-bromo-1-methylpyridin-2(1*H*)-one (2.53 g, 13.5 mmol), and Na_2CO_3 (3.54 g, 33.4 mmol) in DMF (33 mL) and water (15 mL) was added $PdCl_2(dppf){\cdot}CH_2Cl_2$ (543 mg, 0.67 mmol), and the mixture was stirred at 100 °C for 2 h. After cooling at room temperature, the mixture was diluted with EtOAc and water, and filtered through Celite pad. The organic layer of the filtrate was washed with NaCl aqueous solution, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give **17** (1.23 g, 37%) as a pale green solid. ^{1}H NMR ($CDCl_3$) δ 3.51 (s, 3H), 3.78 (s, 3H), 5.32 (s, 2H), 6.58 (d, 1H, J = 9.4 Hz), 7.30 (s, 1H), 7.32–7.50 (m, 6H), 7.70 (d, 1H, J = 2.5 Hz); MS (ESI) m/z 296 $[M+H]^+$.

5.1.24. 5-(3-Hydroxy-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one (18)

The mixture of **17** (1.2 g, 4.06 mmol) and 10% Pd–C (150 mg) in EtOH (20 mL) was stirred at room temperature for 3 days under 3 atm of H_2 atmosphere. The mixture was filtered through Hyflo pad and washed with MeOH/EtOH/$CHCl_3$, and the filtrate was concentrated in vacuo to give **18** (678 mg, 81%) as a colorless solid.

^{1}H NMR (DMSO-d_6) δ 3.43 (s, 3H), 3.62 (s, 3H), 6.40 (d, 1H, J = 9.4 Hz), 7.64 (dd, 1H, J = 9.4, 2.6 Hz), 7.69 (s, 1H), 7.83 (d, 1H, J = 2.6 Hz), 10.26 (br s, 1H); MS (ESI) m/z 206 $[M+H]^+$.

5.1.25. 1-Methyl-5-(1-methyl-3-{[4-(3-methylquinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)pyridin-2(1*H*)-one dihydrochloride (24)

To a suspension of **18** (200 mg, 0.98 mmol) and **23** (292 mg, 1.17 mmol) in toluene (15 mL) was added (tributylphosphoranylidene)acetonitrile (CMBP, 353 mg, 1.46 mmol) and stirred at 100 °C for 10 h before the mixture was concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; 0–5% MeOH in $CHCl_3$, then NH silica gel; 50–80% EtOAc in hexane) to give a free form of the title compound, which was dissolved in a mixture of EtOAc and EtOH, and the mixture was treated with 4 M HCl/EtOAc (0.97 mL). The precipitate was collected by filtration and washed with Et_2O to give **24** (182 mg, 40%) as a pale blue solid. ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H), 3.46 (s, 3H), 3.73 (s, 3H), 5.44 (s, 2H), 6.47 (d, 1H, J = 9.4 Hz), 7.71 (dd, 1H, J = 9.4, 2.6 Hz), 7.75 (d, 2H, J = 8.3 Hz), 7.84 (d, 2H, J = 8.3 Hz), 7.88–7.94 (m, 3H), 8.04 (t, 1H, J = 7.7 Hz), 8.25 (d, 1H, J = 8.1 Hz), 8.35 (d, 1H, J = 8.6 Hz), 9.00 (s, 1H); MS (ESI) m/z 437 $[M+H]^+$; Anal. Calcd for $C_{27}H_{24}N_4O_2 \cdot 1.9HCl \cdot 3H_2O$: C, 57.87; H, 5.74; N, 9.85; Cl, 12.47. Found: C, 58.08; H, 5.77; N, 9.88; Cl, 12.19.

5.1.26. 5-(3-Hydroxy-1-methyl-1*H*-pyrazol-4-yl)-1-methylpiperidin-2-one (19)

The suspension of **18** (285 mg, 1.39 mmol) and PtO_2 (50 mg, 0.22 mmol) in AcOH (10 mL) was stirred at room temperature for 15 h under 3 atm of H_2 atmosphere. The mixture was filtered through Hyflo pad and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–10% MeOH in $CHCl_3$) to give **19** (193 mg, 66%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.72–1.92 (m, 2H), 2.19–2.35 (m, 2H), 2.80 (s, 3H), 2.82–2.90 (m, 1H), 3.15–3.22 (m, 1H), 3.33–3.39 (m, 1H), 3.56 (s, 3H), 7.23 (s, 1H), 9.56 (s, 1H); MS (ESI) m/z 210 $[M+H]^+$.

5.1.27. 1-Methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)piperidin-2-one dihydrochloride (20)

A mixture of **19** (150 mg, 0.72 mmol), 2-[4-(chloromethyl) phenyl]quinoline hydrochloride (208 mg, 0.72 mmol), and K_2CO_3 (248 mg, 1.79 mmol) in DMF (4.2 mL) was stirred at 60 °C for 10 h. After cooling at room temperature, the mixture was partitioned between EtOAc and water. The organic extracts were washed with NaCl aqueous solution and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give a free form of the title compound, which was dissolved in a mixture of EtOH and EtOAc. To the solution was added 4 M HCl/EtOAc. The resulting precipitate was collected by filtration to give **20** (266 mg, 74%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.80–2.00 (m, 2H), 2.22–2.40 (m, 2H), 2.82 (s, 3H), 2.95–3.03 (m, 1H), 3.24–3.30 (m, 1H), 3.39–3.44 (m, 1H), 3.66 (s, 3H), 5.33 (s, 2H), 7.43 (s, 1H), 7.71 (d, 2H, J = 8.4 Hz), 7.81–7.86 (m, 1H), 8.01–8.06 (m, 1H), 8.25 (d, 1H, J = 7.8 Hz), 8.32 (d, 2H, J = 8.4 Hz), 8.38 (d, 1H, J = 8.7 Hz), 8.56 (d, 1H, J = 8.5 Hz), 8.97 (d, 1H, J = 8.7 Hz); MS (ESI) m/z 427 $[M+H]^+$; Anal. Calcd for $C_{26}H_{26}N_4O_2 \cdot 2.3HCl \cdot 1.9H_2O$: C, 57.34; H, 5.94; N, 10.29; Cl, 14.97. Found: C, 57.36; H, 6.04; N, 10.33; Cl, 15.18.

5.1.28. 2-Chloro-3-methylquinoline-6-carbonitrile (26a)

Under argon atmosphere, to a mixture of *N*-(4-cyanophenyl)propanamide (**25a**, 3.61 g, 20.7 mmol) and cetyltrimethylammonium bromide (CTAB, 754 mg, 2.07 mmol) cooled with ice–water bath were added $POCl_3$ (9.5 mL, 104 mmol) and DMF (3.2 mL, 41.5 mmol), and the mixture was stirred at 120 °C for 12 h. The mixture was concentrated in vacuo, and the residue was poured onto ice and diluted with $CHCl_3$. To the mixture was added silica gel and filtered. The filtrate was extracted with $CHCl_3$ for 2 times. The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–20% EtOAc in hexane) to give **26a** (595 mg, 14%) as beige solid. ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H), 7.82 (dd, 1H, J = 8.7, 1.7 Hz), 8.03 (s, 1H), 8.08 (d, 1H, J = 8.5 Hz), 8.16 (d, 1H, J = 1.7 Hz); MS (ESI) m/z 203 $[M+H]^+$.

5.1.29. 2-Chloro-8-fluoro-3-methylquinoline (26b)

Compound **26b** was prepared from **25b** in a manner similar to that described for compound **26a** with a yield of 1.5%. ^{1}H NMR ($CDCl_3$) δ 2.56 (d, 3H, J = 1.0 Hz), 7.34–7.39 (m, 1H), 7.44–7.49 (m, 1H), 7.54 (d, 1H, J = 8.1 Hz), 8.01 (s, 1H); MS (ESI) m/z 196 $[M+H]^+$.

5.1.30. [4-(5-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (27c)

Under argon atmosphere, a mixture of 2-chloro-5-fluoro-3-methylquinoline (**26c**, 443 mg, 2.27 mmol) and **22** (379 mg, 2.49 mmol), and $Pd(PPh_3)_4$ (130 mg, 0.11 mmol) in 1 M Na_2CO_3 aqueous solution (5.7 mL) and DME (15 mL) was stirred at 90 °C for 12 h. The mixture was filtered through Celite pad and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–10% MeOH in $CHCl_3$) to give **27c** (432 mg, 71%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H), 4.61 (d, 2H, J = 5.7 Hz), 5.29 (t, 1H, J = 5.7 Hz), 7.38–7.49 (m, 3H), 7.59–7.63 (m, 2H), 7.66–7.73 (m, 1H), 7.85 (d, 1H, J = 8.5 Hz), 8.38 (s, 1H); MS (ESI) m/z 268 $[M+H]^+$.

5.1.31. 2-[4-(Hydroxymethyl)phenyl]-3-methylquinoline-6-carbonitrile (27a)

Compound **27a** was prepared from **26a** in a manner similar to that described for compound **27c**, with a yield of 76% as a beige solid. ^{1}H NMR ($CDCl_3$) δ 1.93 (t, 1H, J = 5.9 Hz), 2.52 (d, 3H, J = 0.8 Hz), 4.80 (d, 2H, J = 5.9 Hz), 7.49–7.53 (m, 2H), 7.59–7.63 (m, 2H), 7.80 (dd, 1H, J = 8.8 Hz), 8.07 (s, 1H), 8.18–8.21 (m, 2H); MS (ESI) m/z 275 $[M+H]^+$.

5.1.32. [4-(8-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (27b)

Compound **27b** was prepared from **26b** in a manner similar to that described for compound **27c** with a quantitative yield as a yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ 2.04 (br s, 1H), 2.49 (s, 3H), 4.75 (s, 2H), 7.31–7.37 (m, 1H), 7.41–7.48 (m, 3H), 7.56 (d, 1H, J = 8.2 Hz), 7.61 (d, 2H, J = 8.1 Hz), 8.03 (s, 1H); MS (ESI) m/z 268 $[M+H]^+$.

5.1.33. [4-(6-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (27d)

Compound **27d** was prepared from 2-chloro-6-fluoro-3-methylquinoline (**26d**) in a manner similar to that described for compound **27c**, with a yield of 93% as a beige solid. ^{1}H NMR ($CDCl_3$) δ 2.13 (t, 1H, J = 6.0 Hz), 2.46 (s, 3H), 4.77 (d, 2H, J = 6.0 Hz), 7.37–7.49 (m, 4H), 7.54–7.58 (m, 2H), 7.97 (s, 1H), 8.12 (dd, 1H, J = 9.2, 5.4 Hz); MS (ESI) m/z 268 $[M+H]^+$.

5.1.34. [4-(7-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (27e)

Compound **27e** was prepared from 2-chloro-7-fluoro-3-methylquinoline (**26e**) in a manner similar to that described for compound **27c**, with a yield of 87% as a beige solid. ^{1}H NMR ($CDCl_3$) δ 1.95 (br s, 1H), 2.46 (s, 3H), 4.78 (s, 2H), 7.28–7.35 (m, 1H), 7.48 (d, 2H, J = 8.1 Hz), 7.58 (d, 2H, J = 8.1 Hz), 7.64–7.70 (m, 1H), 7.73–7.79 (m, 1H), 8.02 (s, 1H); MS (ESI) m/z 268 $[M+H]^+$.

5.1.35. [4-(3-Ethylquinolin-2-yl)phenyl]methanol (27f)

Compound **27f** was prepared from 2-chloro-3-ethylquinoline (**26f**) in a manner similar to that described for compound **27c**, with a yield of 56% as a beige solid. ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.5 Hz), 2.12 (t, 1H, J = 6.1 Hz), 2.76–2.84 (m, 2H), 4.77 (d, 2H, J = 6.1 Hz), 7.46 (d, 2H, J = 8.3 Hz), 7.51–7.56 (m, 3H), 7.63–7.70 (m, 1H), 7.81 (dd, 1H, J = 8.1, 1.2 Hz), 8.06 (s, 1H), 8.13 (d, 1H, J = 8.4 Hz); MS (ESI) m/z 264 $[M+H]^+$.

5.1.36. [4-(3,6-Dimethylquinolin-2-yl)phenyl]methanol (27g)

Compound **27g** was prepared from **26g** in a manner similar to that described for compound **27c**, with a yield of 87% as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.94 (t, 1H, J = 5.7 Hz), 2.45 (s, 3H), 2.54 (s, 3H), 4.77 (d, 2H, J = 5.7 Hz), 7.45–7.51 (m, 3H), 7.54 (s, 1H), 7.56–7.60 (m, 2H), 7.92 (s, 1H), 8.01 (d, 1H, J = 8.6 Hz); MS (ESI) m/z 264 $[M+H]^+$.

5.1.37. [4-(6-Methoxy-3-methylquinolin-2-yl)phenyl]methanol (27h)

Compound **27h** was prepared from 2-chloro-6-methoxy-3-methylquinoline (**26h**) in a manner similar to that described for compound **27c**, with a yield of 33% as a beige solid. ^{1}H NMR ($CDCl_3$) δ 2.04 (t, 1H, J = 5.7 Hz), 2.45 (s, 3H), 3.94 (s, 3H), 4.76 (d, 2H, J = 5.7 Hz), 7.04 (d, 1H, J = 2.8 Hz), 7.32 (dd, 1H, J = 9.2, 2.8 Hz), 7.46 (d, 2H, J = 8.0 Hz), 7.57 (d, 2H, J = 8.0 Hz), 7.92 (s, 1H), 8.02 (d, 1H, J = 9.2 Hz); MS (ESI) m/z 280 $[M+H]^+$.

5.1.38. 2-[4-(Hydroxymethyl)phenyl]quinoline-3-carbaldehyde (27i)

Compound **27i** was prepared from 2-chloroquinoline-3-carboxaldehyde (**26i**) in a manner similar to that described for compound **27c**, with a yield of 85% as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 4.64 (d, 2H, J = 5.8 Hz), 5.34 (t, 1H, J = 5.8 Hz), 7.53 (d, 2H, J = 8.3 Hz), 7.67–7.75 (m, 3H), 7.94–7.99 (m, 1H), 8.13 (d, 1H, J = 8.4 Hz), 8.28 (d, 1H, J = 8.1 Hz), 8.97 (s, 1H), 10.09 (s, 1H); MS (ESI) m/z 264 $[M+H]^+$.

5.1.39. 3-Methyl-2-[4-({[1-methyl-4-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline-6-carbonitrile (28a)

To a stirred mixture of **27a** (591 mg, 2.15 mmol) in CH_2Cl_2 (12 mL) was added $SOCl_2$ (0.47 mL, 6.44 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was diluted with EtOAc, and the precipitate was collected by filtration to give a 2-[4-(chloromethyl)phenyl]-3-methylquinoline-6-carbonitrile hydrochloride (547 mg, 77%) as a beige solid. A mixture of this beige solid (300 mg, 0.91 mmol), **18** (206 mg, 1.00 mmol) and K_2CO_3 (378 mg, 2.74 mmol) in DMF (6 mL) was stirred at 60 °C for 12 h. After cooling at room temperature, the mixture was diluted with water and stirred for a while. The precipitate was collected by filtration and purified by silica gel column chromatography (0–10% MeOH in CHCl3) to give a yellow solid, which was washed with EtOAc, then EtOH to give **28a** (216 mg, 51%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H), 3.44 (s, 3H), 3.72 (s, 3H), 5.38 (s, 2H), 6.44 (d, 1H, J = 9.4 Hz), 7.62 (d, 2H, J = 8.2 Hz), 7.66–7.72 (m, 3H), 7.85 (s, 1H), 7.89 (d, 1H, J = 2.5 Hz), 7.99 (dd, 1H, J = 8.7, 1.8 Hz), 8.13 (d, 1H, J = 8.7 Hz), 8.39 (s, 1H), 8.60 (d, 1H, J = 1.7 Hz); MS (ESI) m/z 462 $[M+H]^+$; Anal. Calcd for $C_{28}H_{23}N_5O_2 \cdot 0.2H_2O$: C, 72.30; H, 5.07; N, 15.06. Found: C, 72.36; H, 4.92; N, 15.00.

5.1.40. 5-(3-{[4-(3,6-Dimethylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (28g)

To a stirred mixture of **27g** (252 mg, 0.96 mmol) in CH_2Cl_2 (5 mL) was added $SOCl_2$ (0.21 mL, 2.88 mmol), and the mixture as stirred at room temperature for 2 h. The reaction was diluted with EtOAc, and the precipitate was collected by filtration to give 2-[4-(chloromethyl)phenyl]-3,6-dimethylquinoline hydrochloride (245 mg, 81%) as a beige solid. To this beige solid (244 mg, 0.77 mmol) and **18** (173 mg, 0.84 mmol) in DMF was added K_2CO_3 (318 mg, 2.30 mmol), and the mixture was stirred at 60 °C for 12 h. After cooling at room temperature, the mixture was diluted with water and stirred for a while. The precipitate was collected by filtration and purified by silica gel column chromatography (0–10% MeOH in $CHCl_3$) to give a white amorphous solid, which was dissolved in EtOH (5 mL). To the solution was added 4 M HCl/EtOAc (2 mL), and the precipitate was collected by filtration to give **28g** (360 mg, 90%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H), 2.59 (s, 3H), 3.45 (s, 3H), 3.72 (s, 3H), 5.44 (s, 2H), 6.46 (d, 1H, J = 9.4 Hz), 7.70 (dd, 1H, J = 9.4, 2.6 Hz), 7.75 (d, 2H, J = 8.2 Hz), 7.82 (d, 2H, J = 8.2 Hz), 7.87 (s, 1H), 7.88–7.93 (m, 2H), 8.01 (s, 1H), 8.21 (d, 1H, J = 8.8 Hz), 8.89 (1H, br s); MS (ESI) m/z 451 $[M+H]^+$.

5.1.41. 5-(3-{[4-(8-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one trihydrochloride (28b)

Compound **28b** was prepared from **27b** in a manner similar to that described for compound **28g**, with a yield of 26% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.48 (s, 3H), 3.46 (s, 3H), 3.73 (s, 3H), 5.39 (s, 2H), 6.48 (d, 1H, J = 9.4 Hz), 7.50–7.80 (m, 8H), 7.87 (s, 1H), 7.93 (d, 1H, J = 2.5 Hz), 8.36 (br s, 1H); ESI+: 455; Anal. Calcd for $C_{27}H_{23}FN_4O_2 \cdot 2.9HCl \cdot 3.4H_2O$: C, 52.18; H, 5.30; N, 9.01; Cl, 16.54; F, 3.06. Found: C, 52.19; H, 5.06; N, 9.04; Cl, 16.76; F, 3.31.

5.1.42. 5-(3-{[4-(5-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (28c)

Compound **28c** was prepared from **27c** in a manner similar to that described for compound **28g**, with a yield of 64% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.52 (d, 3H), 3.45 (s, 3H), 3.73 (s, 3H), 5.40 (s, 2H), 6.46 (d, 1H, J = 9.4 Hz), 7.49 (dd, 1H, J = 9.6, 7.8 Hz), 7.65 (d, 2H, J = 8.3 Hz), 7.68–7.81 (m, 4H), 7.86 (s, 1H), 7.89–7.94 (m, 2H), 8.55 (br s, 1H); MS (ESI) m/z 455 $[M+H]^+$; Anal. Calcd for $C_{27}H_{23}FN_4O_2 \cdot 2HCl \cdot 2H_2O$: C, 57.55; H, 5.19; N, 9.94; Cl, 12.58; F, 3.37. Found: C, 57.59; H, 5.32; N, 9.94; Cl, 12.61; F, 3.22.

5.1.43. 5-(3-{[4-(6-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (28d)

Compound **28d** was prepared from **27d** in a manner similar to that described for compound **28g**, with a yield of 75% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H), 3.50 (s, 3H), 3.74 (s, 3H), 5.45 (s, 2H), 6.54 (d, 1H, J = 9.4 Hz), 7.73–7.84 (m, 5H), 7.89 (s, 1H), 7.91–8.00 (m, 2H), 8.06 (dd, 1H, J = 9.0, 2.8 Hz), 8.35 (dd, 1H, J = 9.4, 5.0 Hz), 8.89 (s, 1H); MS (ESI) m/z 455 $[M+H]^+$; Anal. Calcd for $C_{27}H_{23}FN_4O_2 \cdot 2HCl \cdot 4H_2O$: C, 54.10; H, 5.55; N, 9.35; Cl, 11.83; F, 3.17. Found: C, 54.28; H, 5.55; N, 9.30; Cl, 11.78; F, 3.16.

5.1.44. 5-(3-{[4-(7-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (28e)

Compound **28e** was prepared from **27e** in a manner similar to that described for compound **28g**, with a yield of 42% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.47 (s, 3H), 3.44 (s, 3H), 3.72 (s, 3H), 5.40 (s, 2H), 6.45 (d, 1H, J = 9.3 Hz), 7.59–7.74 (m, 6H), 7.81 (dd, 1H, J = 9.3, 2.4 Hz), 7.86 (s, 1H), 7.91 (d, 1H, J = 2.4 Hz), 8.14 (dd, 1H, J = 9.1, 6.2 Hz), 8.54 (br s, 1H); MS (ESI) m/z 455 $[M+H]^+$; Anal. Calcd for $C_{27}H_{23}FN_4O_2 \cdot 1.9HCl \cdot 3.8H_2O$: C, 54.76; H, 5.53; N, 9.46; Cl, 11.37; F, 3.21. Found: C, 54.98; H, 5.57; N, 9.29; Cl, 11.08; F, 3.16.

5.1.45. 5-(3-{[4-(3-Ethylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (28f)

Compound **28f** was prepared from **27f** in a manner similar to that described for compound **28g**, with a yield of 44% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, J = 7.5 Hz), 2.83 (q, 2H, J = 7.5 Hz), 3.46 (s, 3H), 3.73 (s, 3H), 5.44 (s, 2H), 6.47 (d, 1H, J = 9.4 Hz), 7.71 (dd, 1H, J = 9.4, 2.6 Hz), 7.75 (d, 2H, J = 8.3 Hz), 7.79 (d, 2H, J = 8.3 Hz), 7.87–7.95 (m, 3H), 8.06 (t, 1H, J = 7.8 Hz), 8.27–8.36 (m, 2H), 9.06 (br s, 1H); MS (ESI) m/z 451 [M+H]$^+$; Anal. Calcd for $C_{28}H_{26}N_4O_2{\cdot}2.3HCl{\cdot}2H_2O$: C, 58.96; H, 5.71; N, 9.82; Cl, 14.30. Found: C, 58.83; H, 5.87; N, 9.77; Cl, 14.38.

5.1.46. 5-(3-{[4-(6-Methoxy-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (28h)

Compound **28h** was prepared from **27h** in a manner similar to that described for compound **28g**, with a yield of 40% as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H), 3.45 (s, 3H), 3.72 (s, 3H), 3.98 (s, 3H), 5.43 (s, 2H), 6.46 (d, 1H, J = 9.4 Hz), 7.63 (d, 1H, J = 2.5 Hz), 7.66–7.77 (m, 4H), 7.80 (d, 2H, J = 8.2 Hz), 7.87 (s, 1H), 7.92 (d, 1H, J = 2.5 Hz), 8.24 (d, 1H, J = 9.4 Hz), 8.83 (br s, 1H); MS (ESI) m/z 467 [M+H]$^+$; Anal. Calcd for $C_{28}H_{26}N_4O_3{\cdot}2HCl{\cdot}2.6H_2O$: C, 57.36; H, 5.71; N, 9.56; Cl, 12.09. Found: C, 57.72; H, 5.87; N, 9.35; Cl, 11.79.

5.1.47. 2-[4-({[1-Methyl-4-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline-3-carbaldehyde (28i)

Compound **28i** was prepared from **27i** in a manner similar to that described for compound **28a**, with a yield of 24% as a dark yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.56 (s, 3H), 3.79 (s, 3H), 5.44 (s, 2H), 6.61 (d, 1H, J = 9.4 Hz), 7.31 (s, 1H), 7.49–7.53 (m, 1H), 7.63–7.68 (m, 3H), 7.71–7.74 (m, 3H), 7.86–7.92 (m, 1H), 8.02 (dd, 1H, J = 8.2, 1.3 Hz), 8.19–8.23 (m, 1H), 8.86 (s, 1H), 10.21 (s, 1H); MS (ESI) m/z 451 [M+H]$^+$.

5.1.48. 5-[3-({4-[3-(Difluoromethyl)quinolin-2-yl]benzyl}oxy)-1-methyl-1*H*-pyrazol-4-yl]-1-methylpyridin-2(1*H*)-one dihydrochloride (29)

To a solution of **28i** (150 mg, 0.33 mmol) in CH_2Cl_2 (3 mL) was added bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor®, 125 mg, 0.57 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with water and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (NH silica gel, (38–70% $CHCl_3$ in hexane) to give a free form of the title compound, which was dissolved in EtOAc. The solution was treated with 4 M HCl/EtOAc, and the precipitate was collected by filtration to give **29** (83 mg, 46%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.47 (s, 3H), 3.73 (s, 3H), 5.40 (s, 2H), 6.50 (d, 1H, J = 9.4 Hz), 7.20 (t, 1H, J = 54 Hz), 7.64–7.79 (m, 6H), 7.88 (s, 1H), 7.93–7.98 (m, 2H), 8.14 (d, 1H, J = 8.5 Hz), 8.26 (d, 1H, J = 7.9 Hz), 8.93 (s, 1H); MS (ESI) m/z 473 [M+H]$^+$; Anal. Calcd for $C_{27}H_{22}F_2N_4O_2{\cdot}2.3HCl{\cdot}2H_2O$: C, 54.74; H, 4.82; N, 9.46; Cl, 13.77; F, 6.41. Found: C, 54.83; H, 4.93; N, 9.53; Cl, 13.73; F, 6.50.

5.1.49. 2-{4-[(4-Iodo-1-methyl-1*H*-pyrazol-3-yl)methoxy]phenyl}-3-methylquinoline (32)

To a solution of (1-methyl-1*H*-pyrazol-3-yl)methanol (**31**, 4.00 g, 35.7 mmol) and 4-(3-methylquinolin-2-yl)phenol (**30**, 8.53 g, 36.3 mmol)in toluene (120 mL) was added CMBP (13.1 g, 54.4 mmol), and the mixture was stirred at 100 °C for 8 h. The mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give a brown syrup, which was dissolved in MeCN (250 mL). To this solution were added CAN (16.0 g, 29.3 mmol) and iodine (7.43 g, 29.3 mmol), and the mixture was stirred at room temperature for 15 min. To the mixture was added iodine (7.43 g, 29.3 mmol), and the mixture was stirred at room temperature for further 30 min. The reaction was concentrated in vacuo, and the residue was diluted with $CHCl_3$ and washed with saturated sodium thiosulfate aqueous solution. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo, and the residue was purified by silica gel column chromatography (20–50% EtOAc in hexane) to give **32** (10.6 mg, 64%) as brown solid. ^{1}H NMR (DMSO-d_6) δ 2.48 (s, 3H), 3.87 (s, 3H), 5.01 (s, 2H), 7.14–7.18 (m, 2H), 7.54–7.64 (m, 3H), 7.67–7.72 (m, 1H), 7.89–7.93 (m, 2H), 7.97 (d, 1H, J = 8.6 Hz), 8.23 (s, 1H); MS (ESI) m/z 456 [M+H]$^+$.

5.1.50. 1-Mmethyl-5-(1-methyl-3-{[4-(3-methylquinolin-2-yl)phenoxy]methyl}-1*H*-pyrazol-4-yl)pyridin-2(1*H*)-one dihydrochloride (33)

To a solution of **32** (6.00 g, 13.2 mmol) in THF (120 mL) cooled with MeOH–ice bath was dropwisely added isopropylmagnesium chloride (2.0 M THF solution; 8.24 mL, 16.5 mmol), and the mixture was stirred at the same temperature for 45 min. To the resultant mixture was added 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (4.30 mL, 21.1 mmol), and the mixture was allowed to stir at room temperature for 2 h. The reaction was quenched with NH_4Cl aqueous solution and extracted with EtOAc. The organic layer was concentrated in vacuo, and the residue was purified by silica gel column chromatography (10–60% EtOAc in hexane, then 30–60% EtOAc in hexane) to give 3-methyl-2-(4-{[1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-3-yl]methoxy}phenyl)quinoline (2.32 g, 39%) as a brown solid. To a mixture of this brown solid (500 mg, 1.10 mmol) and 5-bromo-1-methylpyridin-2(1*H*)-one (413 mg, 2.20 mmol) in DMF (5 mL) and water (1 mL) were added $PdCl_2(dppf){\cdot}CH_2Cl_2$ (54 mg, 0.066 mmol) and Na_2CO_3 (349 mg, 3.29 mmol), and the mixture was stirred at 100 °C for 1 h. The reaction was diluted with water and extracted with EtOAc. The organic layer was concentrated in vacuo, and the residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give a free form of the title compound, which was diluted with EtOH (5 mL). This mixture was treated with 4 M HCl/EtOAc (1.1 mL) and stirred at room temperature for 30 min. The mixture was concentrated in vacuo, and the residue was washed with EtOAc to give **33** (80 mg, 14%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.55 (s, 3H), 3.40 (s, 3H), 3.88 (s, 3H), 5.21 (s, 2H), 6.44 (d, 1H, J = 9.3 Hz), 7.35 (d, 2H, J = 8.8 Hz), 7.58 (dd, 1H, J = 9.3, 2.6 Hz), 7.76–7.82 (m, 3H), 7.88 (t, 1H, J = 7.5 Hz), 7.94 (s, 1H), 8.04 (t, 1H, J = 7.6 Hz), 8.23 (d, 1H, J = 8.1 Hz), 8.30 (d, 1H, J = 8.5 Hz), 8.98 (s, 1H); MS (ESI) m/z 437 [M+H]$^+$; Anal. Calcd for $C_{27}H_{24}N_4O_2{\cdot}1.95HCl{\cdot}0.1C_4H_8O_2{\cdot}1.7H_2O$: C, 60.16; H, 5.56; N, 10.24; Cl, 12.64. Found: C, 60.28; H, 5.89; N, 9.92; Cl, 12.59.

5.1.51. Methyl 1-methyl-4-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-pyrazole-3-carboxylate (36)

To a mixture of 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2(1*H*)-one (**34**, 2.3 g, 9.78 mmol) and methyl 4-iodo-1-methyl-1*H*-pyrazole-3-carboxylate (**35**, 1.25 g, 4.70 mmol) in DMF (40 mL) and water (10 mL) were added $Pd(PPh_3)_4$ (814 mg, 0.71 mmol) and Cs_2CO_3 (3.06 g, 9.40 mmol), and the mixture was stirred at 80 °C for 12 h. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give **36** (812 mg, 70%) as colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.44 (s, 3H), 3.74 (s, 3H), 3.91 (s, 3H), 6.39 (d, 1H, J = 9.3 Hz), 7.51 (dd, 1H, J = 9.4, 2.6 Hz), 7.86 (d, 1H, J = 2.6 Hz), 7.94 (s, 1H); MS (ESI) m/z 248 [M+H]$^+$.

5.1.52. 5-[3-(Hydroxymethyl)-1-methyl-1*H*-pyrazol-4-yl]-1-methylpyridin-2(1*H*)-one (37)

To a mixture of **36** (8.24 g, 33.3 mmol) in THF (200 mL) were added $LiBH_4$ (1.45 g, 66.7 mmol) and EtOH (5.83 mL, 100 mmol), and the mixture was stirred under reflux condition for 2 h. After cooling at room temperature, to the reaction mixture was added 1 M HCl aqueous solution. The mixture was neutralized with $NaHCO_3$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–10% MeOH in $CHCl_3$) to give **37** (3.23 g, 44%) as colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.45 (s, 3H), 3.80 (s, 3H), 4.41 (d, 1H, J = 5.1 Hz), 5.17 (t, 1H, J = 5.1 Hz), 6.43 (d, 1H, J = 9.4 Hz), 7.65 (dd, 1H, J = 9.4, 2.6 Hz), 7.83 (s, 1H), 7.88 (d, 1H, J = 2.6 Hz); MS (ESI) m/z 220 $[M+H]^+$.

5.1.53. 5-[3-(Chloromethyl)-1-methyl-1*H*-pyrazol-4-yl]-1-methylpyridin-2(1*H*)-one hydrochloride (38)

To a mixture of **37** (395 mg, 1.80 mmol) in CH_2Cl_2 (20 mL) was added $SOCl_2$ (0.39 mL, 5.40 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo to give **38** (493 mg, quant) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.47 (s, 3H), 3.83 (s, 3H), 4.80 (s, 2H), 6.47 (d, 1H, J = 9.3 Hz), 7.58 (dd, 1H, J = 9.3, 2.6 Hz), 7.79 (d, 1H, J = 2.6 Hz), 7.87 (s, 1H); MS (ESI) m/z 238, 240 $[M+H]^+$.

5.1.54. 6-Fluoro-3-methyl-2-[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]quinoline (40)

To a mixture of 2-chloro-6-fluoro-3-methylquinoline (**39**, 4.23 g, 21.6 mmol), 4-(2-tetrahydropyranyloxy)phenylboronic acid (5.04 g, 22.7 mmol), and $Pd(PPh_3)_4$ (1.44 g, 1.25 mmol) was added Na_2CO_3 (5.64 g, 53.2 mmol) in DME (80 mL) and water (25 mL), and the mixture was stirred at 100 °C for 16 h. After cooling at room temperature, the mixture was diluted with EtOAc and washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–10% EtOAc in $CHCl_3$) to give a pale brown solid, which was washed with EtOAc to give **40** (6.29 g, 86%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.58–1.77 (m, 3H), 1.87–1.93 (m, 2H), 1.99–2.11 (m, 1H), 2.49 (d, 3H, J = 0.8 Hz), 3.60–3.66 (m, 1H), 3.90–3.97 (m, 1H), 5.50 (t, 1H, J = 3.2 Hz), 7.15–7.19 (m, 2H), 7.35–7.43 (m, 2H), 7.51–7.55 (m, 2H), 7.94 (s, 1H), 8.09 (dd, 1H, J = 9.1, 5.4 Hz); MS (ESI) m/z 338 $[M+H]^+$.

5.1.55. 4-(6-Fluoro-3-methylquinolin-2-yl)phenol (41a)

To a mixture of **40** (6.29 g, 18.6 mmol) in THF (90 mL) was added 1 M HCl aqueous solution (40 mL, 40 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with 1 M NaOH aqueous solution, and the precipitate was collected by filtration to give **41a** (4.72 g, 90%) as pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.46 (d, 3H, J = 0.6 Hz), 6.85–6.91 (m, 2H), 7.46–7.51 (m, 2H), 7.54–7.61 (m, 1H), 7.69 (dd, 1H, J = 9.5, 2.9 Hz), 8.01 (dd, 1H, J = 9.2, 5.5 Hz), 8.19 (s, 1H); MS (ESI) m/z 254 $[M+H]^+$.

5.1.56. 5-(3-{[4-(6-Fluoro-3-methylquinolin-2-yl)phenoxy]methyl}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one (42a)

To a stirred mixture of **41a** (270 mg, 1.07 mmol) and **38** (351 mg, 1.28 mmol) in DMF (7.0 mL) was added K_2CO_3 (369 mg, 2.67 mmol) and stirred at 70 °C for 12 h. After cooling at room temperature, the mixture was diluted with EtOAc, washed with water. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (NH silica gel; 0–20% EtOAc in $CHCl_3$) to give an off-white solid which was washed with EtOAc to give **42a** (390 mg, 81%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.47 (d, 3H, J = 0.6 Hz), 3.38 (s, 3H), 3.88 (s, 3H), 5.13 (s, 2H), 6.44 (d, 1H, J = 9.2 Hz), 7.16–7.20 (m, 2H), 7.56–7.63 (m, 4H), 7.71 (dd, 1H, J = 9.5, 2.9 Hz), 7.77 (d, 1H, J = 2.5 Hz), 7.83 (s, 1H), 8.03 (dd, 1H, J = 9.2, 5.5 Hz), 8.22 (s, 1H); MS (ESI) m/z 455 $[M+H]^+$; Anal. Calcd for $C_{27}H_{23}FN_4O_2{\cdot}0.2H_2O$: C, 70.79; H, 5.15; N, 12.23; F, 4.15. Found: C, 70.59; H, 5.11; N, 12.24; F, 4.21.

5.1.57. 1-Methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)phenoxy]methyl}-1*H*-pyrazol-4-yl)pyridin-2(1*H*)-one (42b)

Compound **42b** was prepared from 4-(quinolin-2-yl)phenol (**41b**) in a manner similar to that described for compound **42a**, with a yield of 53% as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.37 (s, 3H), 3.87 (s, 3H), 5.15 (s, 2H), 6.41–6.45 (d, 1H, J = 9.3 Hz), 7.20–7.25 (m, 2H), 7.54–7.60 (m, 2H), 7.73–7.79 (m, 2H), 7.93 (s, 1H), 7.95–7.99 (m, 1H), 8.03 (d, 1H, J = 8.4 Hz), 8.11 (d, 1H, J = 8.7 Hz), 8.23–8.28 (m, 2H), 8.41 (d, 1H, J = 8.4 Hz); MS (ESI) m/z 423 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O_2$: C, 73.92; H, 5.25; N, 13.26. Found: C, 73.89; H, 5.23; N, 13.27.

5.1.58. (4-Iodo-1-methyl-1*H*-pyrazol-3-yl)methanol (44)

To a solution of 4-iodo-1-methyl-1*H*-pyrazole-3-carboxylic acid (**43**, 719 mg, 2.82 mmol) in THF (7 mL) was added 1,1′-carbonyldiimidazole (CDI; 685 mg, 4.23 mmol), and the mixture was stirred at room temperature for 1 h. To the resultant mixture cooled with ice–water bath was added a mixture of $NaBH_4$ (320 mg, 8.46 mmol) in water (7 mL), and the mixture was stirred at room temperature for 3 h. The reaction was diluted with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give **44** (577 mg, 86%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H), 4.30 (d, 2H, J = 5.3 Hz), 4.93 (t, 1H, J = 5.3 Hz), 7.78 (s, 1H); MS (ESI) m/z 239 $[M+H]^+$.

5.1.59. 2-{4-[(4-Iodo-1-methyl-1*H*-pyrazol-3-yl)methoxy]phenyl}quinoline (45)

To a mixture of **44** (690 mg, 2.90 mmol) in CH_2Cl_2 (7 mL) cooled with ice bath was added $SOCl_2$ (0.32 mL, 4.39 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was concentrated in vacuo. To the residue in DMF (10 mL) were added 4-(quinolin-2-yl)phenol (**41b**, 488 mg, 2.21 mmol) and K_2CO_3 (800 mg, 5.79 mmol), and the mixture was stirred at 70 °C for 8 h. After cooling at room temperature, the residue was partitioned between water and EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (0–5% MeOH in $CHCl_3$) to give **45** (908 mg, 93%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 5.02 (s, 2H), 7.17–7.22 (m, 2H), 7.54–7.69 (m, 1H), 7.73–7.79 (m, 1H), 7.91 (s, 1H), 7.97 (br d, 1H), 8.04 (br d, 1H), 8.11 (d, 1H, J = 8.7 Hz), 8.23–8.28 (m, 2H), 8.41 (br d, 1H); MS (ESI) m/z 442 $[M+H]^+$.

5.1.60. 5-(1-Methyl-3-{[4-(quinolin-2-yl)phenoxy]methyl}-1*H*-pyrazol-4-yl)pyridin-2-ol (46)

Compound **46** was prepared from **45** in a manner similar to that described for compound **13a**, with a yield of 6.0% as a brown solid. ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3H), 5.12 (s, 2H), 6.60 (d, 1H, J = 9.4 Hz), 7.17 (d, 2H, J = 8.9 Hz), 7.41 (s, 1H), 7.47–7.52 (m, 2H), 7.57 (dd, 1H, J = 9.4, 2.7 Hz), 7.68–7.73 (m, 1H), 7.78–7.85 (m, 2H), 8.11–8.20 (m, 4H), 11.61 (br s, 1H); MS (ESI) m/z 409 $[M+H]^+$.

5.1.61. 1-[^{11}C]Methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)phenoxy]methyl}-1*H*-pyrazol-4-yl)pyridin-2(1*H*)-one ([^{11}C]42b)

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction using a Cyclone 18/9 cyclotron (IBA) and [^{11}C]CH_3I was produced from [^{11}C]CO_2 by its reduction with $LiAlH_4$ and subsequent reaction with HI. Generated [^{11}C]CH_3I was passed through a heated

column (200 °C) of silver triflate (AgOTf), and converted to [^{11}C]methyl triflate (CH_3OTf). [^{11}C]CH_3OTf was introduced into a reaction vessel containing **46** (1.2 mg) and 2 mg of NaH (60% oil dispersion, pre-washed with hexane) in a mixed solution of THF (300 μL) and DMSO (25 μL) at −20 °C. The solution was heated at 60 °C for 3 min. After cooling at room temperature, the mixture was transferred to a semi-preparative reverse phase HPLC system (YMC-Pack Pro C_{18} 10 × 250 mm column (YMC Co., Ltd), eluted with 55:45 [0.05 M NH_4OAc–0.1% AcOH]/CH_3CN, eluted at 5.0 mL), which included a solution wash of the reaction vessel with two portions of 70:30 [0.05 M NH_4OAc–0.1% AcOH]/CH_3CN (800, 1000 μL). The fraction containing [^{11}C]**42** was collected in a rotary evaporator including 25% ascorbic acid in dist. water (50 μL) and ethanol (0.5 mL). The solvent was removed under reduced pressure. The residue was dissolved in 10% DMF, 10% PEG400-saline (3.0 mL) and sterile filtered (Millipore GS) to furnish an injectable solution of [^{11}C]**42b**. Analytical chromatography was performed with an Agilent 1200 HPLC system (Agilent Technologies Japan) and an Aloka positron detector RLC-700 (Aloka). Reverse phase chromatography was performed using a YMC-Pack C_{18} Pro column (5 μm, 4.6 × 150 mm; YMC, Kyoto, Japan), eluted with 55:45 [0.05 M NH_4OAc–0.1% AcOH]/CH_3CN at 1 mL/min (Rt = 8 min). [^{11}C]**42b** was identified by comparison with authentic **42b**.

5.2. PDE10A enzyme assay protocol

5.2.1. Cloning and vector construction of PDE10A2

The full-length human PDE10A2 was amplified by PCR using the 1st strand cDNA synthesized from the total RNA isolated from human neuroblastoma TGW cell line. The PCR products were cloned into a pCR2.1-TOPO vector (Invitrogen. Inc.) to confirm sequences. The confirmed plasmid was digested with restricted enzymes, BamHI/HindIII, and this digested product was inserted into a pFastBac1 vector (Invitrogen. Inc.).

5.2.2. Preparation of human PDE10A2 enzyme

Human PDE10A2 enzyme protein was expressed in a *Spodoptera frugiperda* Sf9 insect cell using the Bac-to-Bac Baculovirus Expression System (Invitrogen. Inc.). The infected Sf9 cells were collected by the centrifuge and removed medium. The collected cells were lysed by sonication in the lysis buffer (50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 3 mM DTT, 0.1% NP-40, 20% Glycerol with protease inhibitors). The lysate was centrifuged and supernatant was collected to obtain the PDE10A2 enzyme solution. We confirmed the PDE10A2 expression by Western blot analysis.

5.2.3. PDE10A2 inhibition assay

Inhibition of compounds on human PDE10A enzyme activity was assessed by measuring the amount of cAMP by the Homogeneous Time-Resolved Fluorescence (HTRF) detection method. The assay was performed in 12 μL samples containing a optimal amount of the PDE10A enzyme, a buffer (40 mM Tris–HCl pH 7.5; 5 mM $MgCl_2$), 0.1 μM cAMP and various concentrations of compounds (0.1 nM–10 μM). After compounds were preincubated for 30 min with the enzyme, the reaction was initiated by adding the substrate cAMP and the mixture was incubated for 60 min at room temperature with agitation. The reaction was terminated by the addition of the fluorescence acceptor (cAMP labeled with the dye d2) and the fluorescence donor (anti-cAMP antibody labeled with Cryptate, Cisbio). After 60 min, the fluorescence transfer corresponding to the amount of residual cAMP was measured at lex. 320 nm, lem. 620 nm and lem. 665 nm using an Envision plate reader (PerkinElmer) and signal ratio (665/620) was calculated. The ratio determined in the absence of enzyme was subtracted from all data. The obtained results were converted to activity relative to an uninhibited control (100%) and IC50 values were calculated using Prism software (GraphPad Software, Inc.).

5.3. CYP3A4 inhibitory assay

Time-dependent inhibition assay for CYP3A4 was performed in two steps, a pre-incubation step where the test compound was incubated with human liver microsomes and the secondary incubation period where CYP3A4 substrate, midazolam, was added to the preincubate to measure residual CYP3A4 activity. Midazolam 1′-hydroxylation was used to monitor the CYP3A4 activity.

Each test compound (5 μM) was pre-incubated with human liver microsomes (0.1 mg/mL) and NADPH (1.5 mM) at 37 °C. The pre-incubation times used were 0 and 30 min. Following the pre-incubation step, each compound was co-incubated with midazolam (2 μM) at 37 °C for 20 min. At the end of the incubation, the reaction was terminated by the addition of aqueous solution containing 80% acetonitrile. The concentration of 1′-hydroxymidazolam was determined by LC–MS analysis. The inhibition of CYP3A4 activity was assessed by comparing the amount of 1′-hydroxymidazolam formed in the presence of varying concentrations of inhibitor to the amount of 1′-hydroxymidazolam formed in the solvent control. In each study, a CYP3A4 potent and specific inhibitor, verapamil was used as positive control.

$$\%\ \text{Residual activity} = 100^{*}(\text{Activity NME, 30 min}/\text{Activity vehicle, 30 min}) \quad (1)$$

where Activity NME, 30 min is the activity in the presence of test compound and without pre-incubation, and Activity vehicle, 30 min is the activity in the absence of test compound and with pre-incubation.

5.4. Animal experiments

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Further, the Astellas Pharma Inc. Tsukuba Research Center was awarded Accreditation Status by the AAALAC International. All efforts were made to minimize the number of animals used and to avoid suffering and distress.

5.4.1. In vivo behavioral assay in mice

Phencyclidine-induced hyperlocomotion: ICR mice aged 5–6 weeks were used to evaluate the effect of PDE10A inhibitor on hyper-locomotion induced by the NMDA antagonist phencyclidine (PCP). Immediately after oral administration of either vehicle or agent as pre-treatment, mice were placed into individual plastic test cages (30 × 35 × 17.5 cm) of a SUPERMEX system (PAT.P; Muromachi Kikai Co., Ltd), and measurement of locomotor activity was started. After 1 h, the mice were injected with a post-treatment of saline or PCP (2.5 mg/10 mL/kg, sc), and locomotor activity was measured for a further 60 min. Total locomotor activity for 60 min post-treatment was calculated.

NORT in neonatally PCP-treated mice: Three-day-old male ddY mice were housed 10–12 per cage with a stepmother. Saline or PCP (15 mg/kg) was administered subcutaneously once daily on days 7, 9, and 11 after birth. The mice were separated from their mother at 3 weeks of age, and used for NORT at 8–9 weeks old. Neonatal mice were treated with PCP, and the NORT was conducted as previously described.[19]

5.4.2. Mouse pharmacokinetic study

The ICR mice were treated orally with compound **42b** or MP-10 (3.0 mg/kg) suspended in 0.5% methylcellulose aqueous solution. Whole brain samples were collected at 1 h after administration,

and stored at −20 °C and homogenized in 4-fold volume of phosphate buffered saline (pH 7.4) before extraction processing. Extraction and analysis of compound concentrations were performed via LC–MS/MS with a ACQUITY UPLC(Waters) and Xevo TQ(Waters).

5.4.3. [^{11}C]42b distribution study

Mice brain distribution study: 8 weeks old ddY mice received [^{11}C]**42b** injection via the tail vein (approximately 7 MBq in each mice) at 60 min prior to sacrifice. Striatum and cerebellum were isolated immediately following sacrifice. Brain samples were weighed and measured for radioactivity using a γ-counter (Wallac 2480, Perkin Elmer, Waltham, MA, USA). The data obtained from the brain in units of Bq/g were converted to SUV using the following equation:

$$\mathrm{SUV} = \frac{\text{Brain region radioactivity (Bq/g)}}{\text{Injected radioactivity per body weight (Bq/g)}}$$

Rat PET imaging study: PET scans were conducted using an Inveon Multimodality system (Siemens, Knoxville, TN, USA). PET emission scans were performed with 8 weeks old SD rats under anesthesia induced by 2.5% isoflurane. [^{11}C]**42b** (approximately 50 MBq) was bolus intravenously injected to rats treated with MP-10 (10 mg/kg, iv) or saline at 10 min before radioligand administration. And then, dynamic PET emission data were collected for 60 min. Data from PET acquisition was reconstructed using ASIproVM software (Siemens).

5.5. Human liver microsomal assays

Pooled human liver microsomes (Xenotech LLC.) were diluted in 100 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.4) containing 0.1 mM EDTA. The incubation mixtures (270 μL total volume), which contained 0.2 mg/mL of microsomal proteins, and 1 mM NADPH (30 μL) were pre-incubated for 5 min at 37 °C. Reactions were initiated by the addition of 0.2 μM of substrates. After the appropriate incubation time (0, 15, 30, and 45 min), 50 μL of incubation mixture was transferred into 80% acetonitrile containing internal standard (50 ng/mL diazepam, 250 μL) and centrifuged for 10 min at 2800 rpm. The supernatant (200 μL) was prepared and analyzed via LC–MS/MS with a Surveyor HPCL system (Thermo Fisher Scientific Inc.) and TSQ Quantum ultra tandem triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc.). The in vitro intrinsic clearance (CLint, vitro) was calculated using Eq. 2, which is based on the time course of the residual ratio of the compounds.[20]

$$\text{CLint, vitro (mL/min/kg)} = \frac{\text{Ke}(1/\text{min}) \times \text{MS content (mg/kg)}}{\text{MS Protein Concd (mg/mL)}} \quad (2)$$

where Ke is the disappearance rate constant.

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